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(71) Applicant: INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors: KENNEDY, Neil, F.; 6571 Jeremie Drive, San Jose, CA 95120 (US). GUEGLER, Karl, J.; 1048 Oakland Avenue, Menlo Park, CA 94025 (US). SEILHAMER, Jeffrey, J.; 12555 La Cresta, Los Altos Hills, CA 94022 (US).

(74) Agent: GLAISTER, Debra, J.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).

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(54) Title: HUMAN HOMOLOG OF THE MOUSE RAB18 GENE

(57) Abstract

The present invention provides nucleotide and amino acid sequences that identify and encode a human homolog of mouse RAB18 (HRAB18) expressed in human pituitary. The present invention also provides for antisense molecules to the nucleotide sequences which encode HRAB18, hybridization probes or oligonucleotides for the detection of HRAB18-encoding nucleotide sequences, and a diagnostic test based on HRAB18-encoding nucleic acid molecules. The present invention further provides for genetically engineered host cells for the expression of HRAB18, biologically active HRAB18, antibodies capable for binding specifically to HRAB18, and treatment methods comprising administration of compounds capable of binding HRAB18.

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ATG GAC GAG GAC GTG CTA ACC ACC CTG AAG ATC CTC ATC ATC GGC GAG      48
Met Asp Glu Asp Val Leu Thr Thr Leu Lys Ile Leu Ile Ile Gly Glu
  1               5                  10                  15

AGT GGG GTG GGC AAG TCC AGC CTG CTC TTG AGG TTC ACA GAT GAT ACG      96
Ser Gly Val Gly Lys Ser Ser Leu Leu Leu Arg Phe Thr Asp Asp Thr
            20                  25                  30

TTT GAT CCA GAA CTT GCA GCA ACA ATA GGT GTT GAC TTT AAG GTG AAA     144
Phe Asp Pro Glu Leu Ala Ala Thr Ile Gly Val Asp Phe Lys Val Lys
        35                  40                  45

ACA ATT TCA GTG GAT GGA AAT AAG GCT AAA CTT GCA ATA TGG GAT ACT     192
Thr Ile Ser Val Asp Gly Asn Lys Ala Lys Leu Ala Ile Trp Asp Thr
    50                  55                  60

GCT GGT CAA GAG AGG TTT AGA ACA TTA ACT CCC AGC TAT TAT AGA GGT     240
Ala Gly Gln Glu Arg Phe Arg Thr Leu Thr Pro Ser Tyr Tyr Arg Gly
65                  70                  75                  80

GCA CAG GGT GTT ATA TTA GTT TAT GAT GTC ACA AGA AGA GAT ACA TTT     288
Ala Gln Gly Val Ile Leu Val Tyr Asp Val Thr Arg Arg Asp Thr Phe
                85                  90                  95

GTT AAA CTG GAT AAC TGG TTA AAT GAA TTG GAA ACA TAC TGT ACA AGA     336
Val Lys Leu Asp Asn Trp Leu Asn Glu Leu Glu Thr Tyr Cys Thr Arg
            100                 105                 110

AAT GAC ATA GTA AAC ATG CTA GTT GGA AAT AAA ATC GAT AAG GAA AAT     384
Asn Asp Ile Val Asn Met Leu Val Gly Asn Lys Ile Asp Lys Glu Asn
        115                 120                 125

CGT GAA GTC GAT AGA AAT GAA GGC CTG AAA TTT GCA CGA AAG CAT TCC     432
Arg Glu Val Asp Arg Asn Glu Gly Leu Lys Phe Ala Arg Lys His Ser
    130                 135                 140

ATG TTA TTT ATA GAG GCA AGT GCA AAA ACC TGT GAT GGT GTA CAA TGT     480
Met Leu Phe Ile Glu Ala Ser Ala Lys Thr Cys Asp Gly Val Gln Cys
145                 150                 155                 160

GCC TTT GAA GAA CTT GTT GAA AAG ATC ATT CAG ACC CCT GGA CTG TGG     528
Ala Phe Glu Glu Leu Val Glu Lys Ile Ile Gln Thr Pro Gly Leu Trp
                165                 170                 175

GAA AGT GAG AAC CAG AAT AAA GGA GTC AAA CTG TCA CAC AGG GAA GAA     576
Glu Ser Glu Asn Gln Asn Lys Gly Val Lys Leu Ser His Arg Glu Glu
            180                 185                 190

GGC CAA GGA GGA GGA GCC TGT GGT GGT TAT TGC TCT GTG TTA TAA         621
Gly Gln Gly Gly Gly Ala Cys Gly Gly Tyr Cys Ser Val Leu *
        195                 200                 205
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# HUMAN HOMOLOG OF THE MOUSE RAB18 GENE

## TECHNICAL FIELD

The present invention is in the field of molecular biology; more particularly, the present invention describes the nucleic acid and amino acid sequences of a human homolog of the mouse rab18 gene.

## BACKGROUND ART

RAB proteins belong to the RAS superfamily of G proteins that comprises nearly 50 related monomeric GTPases with molecular weights between about 20,000 to 30,000. Monomeric G proteins may interact with several types of effector proteins to trigger specific cellular responses. RAB proteins act as specific regulators of intracellular membrane trafficking, exocytosis, and endocytosis to control vesicle budding, targeting and fusion. RAS proteins activate a cascade of serine/threonine protein kinases to regulate cell growth and differentiation. RHO and RAC proteins are involved in relaying signals from cell-surface receptors to the actin cytoskeleton. (Alberts, B et al. Molecular Biology of the Cell, 3rd ed, Garland Publishing, Inc., New York City , NY (1994)).

G proteins exist in equilibrium between two forms, a GTP-bound form which is active and interacts with effector proteins, and a GDP-bound form which is inactive. The distribution of active and inactive G proteins appears to be modulated in part by certain regulatory proteins that affect the rates of GDP release or GTP hydrolysis by G proteins. For example, guanine nucleotide release proteins (GNRPs) catalyze the release of bound GDP. Subsequently GTP binds to the nucleotide binding site, and the G protein is activated. Alternatively, GTPase-activating proteins (GAPs) increase the rate of hydrolysis of GTP with concomitant production of GDP and phosphate. The GDP remains bound to the G protein and inactivates the protein. Other G proteins interact with a guanine nucleotide dissociation inhibitor (GDI) that inhibits the release of GDP (Barangar (1994) J Biol Chem 269:13637-43).

Most information on monomeric G proteins has been obtained by studying the structure and function of RAS proteins as described in Hesketh R, The Oncogene FactsBook, Academic Press, Great Britain (1995). Much less is known about the structural features important for the activity of other members of the RAS superfamily.

### RAB Proteins

So far, over 30 RAB proteins have been identified in mammalian cells with sequences that share between 35% and 95% identity indicating a broad range of functional specificities. Usually, these proteins have been localized to specific organelles. For example, RAB1 is localized to the ER

and Golgi complex, RAB2 in the transitional ER and the cis Golgi network, RAB3 to secretory vesicles, RAB4 to early endosomes, RAB5 to early endosomes and the plasma membrane, RAB6 to medial and trans Golgi cisternae, RAB7 to late endosomes, and RAB9 to late endosomes and the trans Golgi network (Alberts, supra).

In addition, RAB proteins are localized to specific tissue types. For example, RAB17 is found in epithelial cells which contains distinct apical, basolateral, and transcytotic transport pathways. Isoforms of RAB3 with about 77-85% homology appear to be largely restricted to cell lineages containing regulated secretory pathways, such as neurons, endocrine, and exocrine cells (Fischer von Mollard (1994) J Biol Chem 269: 10971-74). RAB18 is found to be expressed at a high level in the mouse brain, at a moderate level in the pituitary gland, and at low levels in the liver. This protein may play a role in secretory vesicle recycling (Yu H et al (1993) Gene 132:273-8).

Both RAB and RAS proteins appear to share conserved domains which are involved in guanine nucleotide binding or are involved in the conformational changes associated with GTP binding and GTP hydrolysis. Characteristic structural motifs associated with the GTP binding site include a first motif, $GX_4GK(S/T)$, which interacts with the alpha and beta phosphates of GDP or GTP. Another motif, DXXG, also appears to interact with the gamma phosphate. A third motif, (N/T)(K/Q)XD, interacts with the guanine ring. A tightly bound $Mg^+$ is coordinated to a conserved threonine residue and to the beta and gamma phosphate groups of GTP. In addition the $Mg^+$ interacts with the serine/threonine residue of the first motif, and with the invariant aspartate of the third motif. Domains that appear important for conformational changes include the effector L2 loop and the helix a2/loop5 (a2L5) which appear to be involved in interactions with specific GEPs and GAPs (Ferro-Novick S. (1993) Ann. Rev. Cell Biol. 9:575-99).

In addition, posttranslational modification by a lipid moiety is critical for membrane localization and the proper activity of RAB. This modification occurs at the C-terminal end of the RAB proteins whereby a geranylgeranyl (GG) moiety, a 20-carbon isoprene unit, is usually attached via a thioether bond to one of two cysteine residues. Most RAB proteins have C termini that end in -XXCC (35%), -XCXC (37%), -CCXX (15%), -CCXXX (8%) and -CXXX (5%). Some RAB proteins, such as RAB3A, that have the -XCXC motif appear to be geranylgeranylated on each of the adjacent cysteine residues. (Farnsworth (1994) Proc Natl Acad Sci USA 91: 11963-7). This modification reaction appears to involve a single RAB-specific geranylgeranyltransferase (RAB GGTase II) that transfers the lipid moieties to the different RAB

motifs. A RAB escort protein (REP) additionally participates in the lipidation reaction by binding the protein substrate, and then by forming a complex with RAB GGTase II. Then, the GGTase II transfers the geranylgeranyl moiety from geranylgeranylpyrophosphate to the protein substrate.

RAB proteins' mode of action as regulators of membrane trafficking between intracellular compartments is not well understood. It has been proposed that RAB proteins cycle between soluble and membrane-bound forms and interact with vesicular and target membrane-bound proteins. When a RAB protein is bound by GDP (i.e., in an inactive form), it exists in a conformation in which its lipid moiety is hidden within the protein. Therefore, the protein remains in soluble form. Once the RAB protein is activated by a GNRP, the GDP is exchanged for GTP which alters the conformation of the protein so that the lipid moiety remains exposed and RAB becomes membrane-bound.

Membrane-bound RAB with GTP at the nucleotide binding site is localized where membrane vesicles are being pinched off and binds with a complex of certain vesicle specific proteins (v-SNARE). The RAB protein remains bound to the vesicle surface until the vesicle docks at the target membrane at which time the v-SNARE interacts with target associated SNARE (t-SNARE). At this time the GTP bound to RAB is hydrolyzed to GDP. Concomitantly, RAB alters its conformation so that its lipid moiety no longer is exposed and RAB is released from the target-membrane surface. Therefore, it appears that SNARE complexes may serve as the ultimate targets of regulation by RAB (Alberts, supra).

## DISCLOSURE OF THE INVENTION

The present invention relates to polynucleotides and polypeptides of a human homolog of mouse RAB18 designated herein as HRAB18. The present invention also provides for HRAB18 antisense DNA and expression vectors and host cells comprising polynucleotides encoding HRAB18.

Furthermore, the subject invention provides a method for producing HRAB18, and a purified HRAB18 polypeptide having the sequence shown in SEQ ID NO:2.

The subject invention also relates to diagnostic tests and compositions for the detection of disorders associated with altered expression of HRAB18, and more particularly, disorders associated with the pituitary gland.

A method of screening a plurality of test compounds to identify compounds binding to HRAB18 is also proposed along with their use as therapeutic compounds for the treatment of disorders related to the altered expression of HRAB18.

## BRIEF DESCRIPTION OF DRAWINGS

Figure 1 displays the nucleotide sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) for HRAB18 found in Incyte clone 112352.

Figure 2 shows the amino acid alignment of HRAB18 with mouse RAB18. Alignments shown were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison WI).

Figure 3 shows a hydrophobicity plot for the amino acid sequence of HRAB18 using the hydrophobicity program of DNASTAR.

## MODES FOR CARRYING OUT THE INVENTION

**Definitions**

As used herein, the term "human homolog of mouse rab18" or "HRAB18" refers to the polypeptide as shown in SEQ ID NO:2. Polynucleotide sequences encoding HRAb18 were found in a human pituitary cDNA library. HRAB18 is a member of the RAB subfamily of monomeric G proteins and may be involved in the regulation of secretory vesicle recycling. In one embodiment disclosed herein, HRAB18 is encoded by the polynucleotide shown in SEQ ID NO:1 beginning with nucleotide 45 and ending with nucleotide 664. The present invention also relates to the upstream and downstream sequences shown in SEQ ID NO:1, that is, nucleotides 1 to 44 and 665 to 1148 in SEQ ID NO:1 which may affect mRNA transcript stability. HRAB18 may be naturally occurring, recombinantly produced or chemically synthesized. Also included within the scope of the present invention are active fragments of HRAB18. As used herein, the lower case "hrab18" refers to a nucleic acid sequence while the upper case "HRAB18" refers to a protein, peptide or amino acid sequence.

"Active" refers to those forms of HRAB18 which retain the biologic and/or immunologic activities of naturally occurring HRAB18.

"Naturally occurring HRAB18" refers to HRAB18 produced by human cells that have not been genetically engineered and specifically contemplates various HRAB18 forms arising from post-translational modifications of the polypeptide including but not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

"Derivative" refers to polypeptides derived from naturally occurring HRAB18 by chemical modifications such as ubiquitination, labeling (e.g., with radionuclides, various enzymes, etc.), pegylation (derivatization with polyethylene glycol) or by insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

"Recombinant variant" refers to any polypeptide differing from naturally occurring HRAB18 by amino acid insertions, deletions, and substitutions, created using recombinant DNA techniques. Guidance in

determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest may be found by comparing the sequence of the particular HRAB18 with that of other RAB proteins and minimizing the number of amino acid sequence changes made in regions of high homology.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine, i.e., conservative amino acid replacements. "Insertions" or "deletions" are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in HRAB18 using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Where desired, a "signal or leader sequence" can direct the HRAB18 polypeptide through the membrane of a cell. Such a sequence may be naturally present on the HRAB18 polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, often at least about 7 amino acids, typically at least about 9 to 13 amino acids, and, in various embodiments, at least about 17 or more amino acids. To be active, HRAB18 polypeptides must have sufficient length to display biologic and/or immunologic activity.

An "oligonucleotide" or polynucleotide "fragment", "portion," or "segment" is a stretch of nucleotide residues which is long enough to use in polymerase chain reaction (PCR) or various hybridization procedures to identify or amplify HRAB18 mRNA or DNA molecules.

The present invention includes purified HRAB18 polypeptides from natural or recombinant sources, ie, cells transformed with recombinant nucleic acid molecules encoding HRAB18. Various methods for the isolation of the HRAB18 polypeptides may be accomplished by procedures well known in the art. For example, such polypeptides may be purified by immunoaffinity chromatography by employing the antibodies provided by the present invention. Various other methods of protein purification well known in the art include those described in Deutscher M (1990) Methods in Enzymology, Vol 182, Academic Press, San Diego; and Scopes R (1982) Protein Purification: Principles and Practice. Springer-Verlag, NYC, both incorporated herein by reference.

"Recombinant" refers to a polynucleotide which encodes HRAB18 and is

prepared using recombinant DNA techniques. The DNA which encodes HRAB18 may also include allelic or recombinant variants and mutants thereof.

"Oligonucleotides" or "nucleic acid probes" are prepared based on the cDNA sequence which encodes HRAB18 (SEQ ID NO:2). Oligonucleotides comprise portions of the DNA sequence having between 10 and 60 nucleotides and preferably between 15 nucleotides and 60 nucleotides. Nucleic acid probes comprise portions of the sequence having fewer nucleotides than about 6 kb, usually fewer than about 1 kb. In one embodiment of the present invention, the oligonucleotide probes will comprise sequence that is identical or complementary to a portion of HRAB18 where there is little or no identity or complementarity with any known or prior art molecule. After appropriate testing to eliminate false positives, these probes may be used to determine whether mRNA encoding HRAB18 is present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh PS et al (1992 PCR Methods Appl 1:241-250).

Probes may be derived from naturally occurring or recombinant single- or double-stranded nucleic acids or be chemically synthesized. They may be labeled by nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook J et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons, NYC, both incorporated herein by reference.

Alternatively, recombinant variants encoding HRAB18 may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations may also be introduced to modify the properties of the polypeptide, to change ligand-binding affinities, interchain affinities, or polypeptide degradation or turnover rate.

The present invention, in one aspect, provides a nucleotide sequence identified in Incyte 112352 encoding HRAB18, a human homolog of mouse rab18 gene. In another aspect, the present invention provides purified HRAB18 polypeptide from natural or recombinant sources. The amino acid sequence is shown in SEQ ID NO:2.

One embodiment of the subject invention is to provide for hrab18-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences encoding HRAB18. Further embodiments of the present invention are cells transformed with recombinant

nucleic acid molecules encoding HRAB18 and antibodies to HRAB18.

Polynucleotides, polypeptides and antibodies to HRAB18 may be useful in diagnostic assays for detection of disorders of the regulation of intermembrane trafficking, such as, for example, endocytosis or exocytosis and as diagnostic compositions for the detection of disorders of secretory tissue, particularly neuronal and pituitary tissue. Additionally, these diagnostic tools may be useful in diagnosing disorders associated with tissue damage.

The nucleotide sequence encoding HRAB18 has numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use in the construction of oligomers for PCR, use for chromosome and gene mapping, use in the recombinant production of HRAB18, and use in generation of anti-sense DNA or RNA, their chemical analogs and the like. Uses of nucleotides encoding HRAB18 disclosed herein are exemplary of known techniques and are not intended to limit their use in any technique known to a person of ordinary skill in the art. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known (eg, the triplet genetic code, and specific base pair interactions).

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of HRAB18-encoding nucleotide sequences, some bearing minimal homology to the nucleotide sequence of any known and naturally occurring gene may be produced. The invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HRAB18, and all such variations are to be considered as being specifically disclosed.

Although the nucleotide sequences which encode HRAB18 and/or its variants are preferably capable of hybridizing to the nucleotide sequence of naturally occurring HRAB18 under stringent conditions, it may be advantageous to produce nucleotide sequences encoding HRAB18 or its derivatives possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HRAB18 and/or its

derivatives without altering the encoded amino acid sequence include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

Nucleotide sequences encoding HRAB18 may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA techniques (Sambrook J et al. supra). Useful nuclectide sequences for joining to hrab18 include an assortment of cloning vectors, e.g., plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Vectors of interest include expression vectors, replication vectors, probe generation vectors, sequencing vectors, and the like. In general, vectors of interest may contain an origin of replication functional in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for the host cell.

The subject invention provides for hrab18-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences encoding HRAB18. Such probes may also be used for the detection of other rab gene encoding sequences and should preferably contain at least 50% of the nucleotides from the conserved region or active site. The hybridization probes of the subject invention may be derived from the nucleotide sequences of the SEQ ID NO:1 or from genomic sequences including promoters, enhancers and/or possible introns of respective naturally occurring hrab18 polynucleotides. Hybridization probes may be labeled by a variety of reporter groups, including radionuclides such as $^{32}P$ or $^{35}S$, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

In addition, the subject invention provides for nucleic hybridization probes capable of hybridizing with either upstream or downstream sequences that may play a role in HRAB18 translation. Such probes may also be used to detect similar regulatory sequences for polypeptide translation.

PCR, as described US Patent Nos. 4,683,195; 4,800,195; and 4,965,188, provides additional uses for oligonucleotides based upon the nucleotide sequence which encodes HRAB18. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both and comprise a discrete nucleotide sequence for diagnostic use or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Full length genes may be cloned from known sequence using a new method which employs XL-PCR (Perkin-Elmer, Foster City, CA) to amplify long pieces of DNA. This method was developed to allow a single researcher to process multiple genes (up to 20 or more) at a time and to obtain an extended

(possibly full-length) sequence within 6-10 days. It replaces current methods which use labelled probes to screen libraries and allow one researcher to process only about 3-5 genes in 14-40 days.

In the first step, which can be performed in about two days, primers are designed and synthesized based on a known partial sequence. In step 2, which takes about six to eight hours, the sequence is extended by PCR amplification of a selected library. Steps 3 and 4, which take about one day, are purification of the amplified cDNA and its ligation into an appropriate vector. Step 5, which takes about one day, involves transforming and growing up host bacteria. In step 6, which takes approximately five hours, PCR is used to screen bacterial clones for extended sequence. The final steps, which take about one day, involve the preparation and sequencing of selected clones. If the full length cDNA has not been obtained, the entire procedure is repeated using either the original library or some other preferred library.

The preferred library may be one that has been size-selected to include only larger cDNAs or may consist of single or combined commercially available libraries, eg. lung, liver, heart and brain from Gibco/BRL (Gaithersburg MD). The cDNA library may have been prepared with oligo dT or random primers. The advantage of using random primed libraries is that generally have more sequences which contain 5' ends of genes. A randomly primed library may be particularly useful if an oligo dT library does not yield a complete gene. Obviously, the larger the protein, the less likely it is that the complete gene will be found in a single plasmid.

Other means of producing specific hybridization probes for hrab18 DNAs include the cloning of nucleic acid sequences encoding HRAB18 or HRAB18 derivatives into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides.

It is now possible to produce a DNA sequence, or portions thereof, encoding HRAB18 and their derivatives entirely by synthetic chemistry, after which the gene can be inserted into any of the many available DNA vectors using reagents, vectors and cells that are known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into the hrab18 sequences or any portion thereof. The nucleotide sequence of hrab18 sequences can be confirmed through DNA sequencing techniques.

Methods for DNA sequencing are well known in the art. Conventional

enzymatic methods employed DNA polymerase Klenow fragment, SEQUENASE® (US Biochemical Corp, Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single- and double-stranded templates. The chain termination reaction products were electrophoresed on urea-acrylamide gels and detected either by autoradiography (for radionuclide-labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day (using machines such as the Catalyst 800 and the Applied Biosystems 377 or 373 DNA sequencer). Alternatively, cDNA inserts may be sequenced using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) along with Applied Biosystems 377 or 373 DNA Sequencing System.

The nucleotide sequence can be used in an assay to detect disorders associated with altered expression of HRAB18. The nucleotide sequence can be labeled by methods known in the art and added to a fluid or tissue sample from a patient under hybridizing conditions. After an incubation period, the sample is washed with a compatible fluid which optionally contains a dye (or other label requiring a developer) if the nucleotide has been labeled with an enzyme. After the compatible fluid is rinsed off, the dye is quantitated and compared with a standard. If the amount of dye is significantly elevated, the nucleotide sequence has hybridized with the sample, and the assay indicates the presence of membrane trafficking disorders.

The nucleotide sequence for hrab18 can be used to construct hybridization probes for mapping that gene. The nucleotide sequence provided herein may be mapped to a particular chromosome or to specific regions of that chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include in situ hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries, flow-sorted chromosomal preparations, or artificial chromosome constructions YAC or P1 constructions. The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York City.

Fluorescent in situ hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of

hrab18 on a physical chromosomal map and a specific disease (or predisposition to a specific disease) can help delimit the region of DNA associated with that genetic disease. The nucleotide sequence of the subject invention may be used to detect differences in gene sequence between normal and carrier or affected individuals.

Nucleotide sequences encoding HRAB18 may be used to produce purified HRAB18 using well known methods of recombinant DNA technology. Among the many publications that teach methods for the expression of genes after they have been isolated is Goeddel (1990) Gene Expression Technology, Methods and Enzymology, Vol 185, Academic Press, San Diego CA. Purification steps vary with the production process and the particular protein produced. Various methods for the isolation of the HRAB18 polypeptides may be accomplished by procedures well known in the art including those described in Deutscher M (1990) Methods in Enzymology, Vol 182, Academic Press, San Diego CA; and Scopes R (1982) Protein Purification: Principles and Practice, Springer-Verlag, New York City, both incorporated herein by reference.

HRAB18 may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species in which HRAB18 nucleotide sequences are endogenous or from a different species. Advantages of producing HRAB18 by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

Cells transformed with DNA encoding HRAB18 may be cultured under conditions suitable for the expression of RAB proteins and recovery of the protein from the cell culture. HRAB18 produced by a recombinant cell may be secreted or may be contained intracellularly, depending on the hrab18 sequence and the genetic construction used. In general, it is more convenient to prepare recombinant proteins in secreted form.

In addition to recombinant production, fragments of HRAB18 may be produced by direct peptide synthesis using solid-phase techniques (Stewart et al (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco, CA; Merrifield J (1963) J Am Chem Soc 85:2149-2154. In vitro protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Foster City, CA) in accordance with the instructions provided by the manufacturer. Various fragments of HRAB18 may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

HRAB18 for antibody induction does not require biological activity; however, the protein must be antigenic. Peptides used to induce specific

antibodies may have an amino acid sequence consisting of at least five amino acid residues, preferably at least 10 amino acid residues. They should mimic a portion of the amino acid sequence of the protein and may contain the entire amino acid sequence of a small naturally occurring molecule such as HRAB18. Short stretches of HRAB18 may be fused with those of another protein such as keyhole limpet hemocyanin (KLH, Sigma, St Louis, MO) and the chimeric molecule used for antibody production.

Antibodies specific for HRAB18 may be produced by inoculation of an appropriate animal with the polypeptide or an antigenic fragment. An antibody is specific for HRAB18 if it is produced against an epitope of the polypeptide and binds to at least part of the natural or recombinant protein. Antibody production includes not only the stimulation of an immune response by injection into animals, but also analogous steps in the production of synthetic antibodies or other specific-binding molecules such as the screening of recombinant immunoglobulin libraries (Orlandi R et al (1989) Proc. Nat. Acad. Sci. USA 86:3833-3837, or Huse WD et al (1989) Science 256:1275-1281) or the in vitro stimulation of lymphocyte populations. Current technology (Winter G and Milstein C (1991) Nature 349:293-299) provides for a number of highly specific binding reagents based on the principles of antibody formation. These techniques may be adapted to produce molecules specifically binding HRAB18.

The present invention includes purified HRAB18 polypeptide from natural or recombinant sources, ie, cells transformed with recombinant nucleic acid molecules encoding HRAB18. Various methods for the isolation of the HRAB18 polypeptides may be accomplished by procedures well known in the art. For example, such polypeptides may be purified by immunoaffinity chromatography by employing the antibodies provided by the present invention. Various other methods of protein purification well known in the art include those described in Deutscher M (1990) Methods in Enzymology, Vol 182, Academic Press, San Diego, CA; and Scopes R (1982) Protein Purification: Principles and Practice, Springer-Verlag, New York City, both incorporated herein by reference.

HRAB18 may be used to screen or design drugs that may be employed to regulate hormonal secretions or the expression of specific receptors of the pituitary that are associated with abnormal expression of HRAB18. Alternatively, HRAB18 itself may serve to control excessive hormonal secretion or to regulate the expression of specific receptors. Additionally, HRAB18 may serve similar functions in other neuronal tissues, particularly where secretory pathways play an important role in function.

HRAB18 as a bioactive agent or composition may be administered in a suitable therapeutic dose determined by any of several methodologies

including clinical studies on mammalian species to determine maximal tolerable dose and on normal human subjects to determine safe dose. Additionally, the bioactive agent may be complexed with a variety of well established compounds or compositions which enhance stability or pharmacological properties such as half-life. It is contemplated that the therapeutic, bioactive composition may be delivered by intravenous infusion into the bloodstream or any other effective means which could be used for treating problems involving the altered expression or activity of RAB proteins.

The examples below are provided to illustrate the subject invention. These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

**INDUSTRIAL APPLICABILITY**

**I Isolation of mRNA and Construction of cDNA Libraries**

Incyte clone 112352 was identified among the sequences of a human pituitary cDNA library constructed from a pooled sample of 21 whole, normal human pituitary glands from brains of Caucasian males and females with a range of ages from 16-70 years. Poly $A^+$ RNA was isolated using biotinylated oligo d(T) primer and streptavidin coupled to a paramagnetic particle (Promega Corp, Madison WI) and sent to Stratagene (La Jolla, CA).

Stratagene prepared the cDNA library using oligo d(T) priming. Synthetic adapter oligonucleotides were ligated onto the cDNA molecules enabling them to be inserted into the Uni-ZAP™ vector system (Stratagene). This allowed high efficiency unidirectional (sense orientation) lambda library construction and the convenience of a plasmid system with blue/white color selection to detect clones with cDNA insertions.

The quality of the cDNA library was screened using DNA probes, and then, the pBluescript® phagemid (Stratagene) was excised. This phagemid allows the use of a plasmid system for easy insert characterization, sequencing, site-directed mutagenesis, the creation of unidirectional deletions and expression of fusion polypeptides. Subsequently, the custom-constructed library phage particles were infected into E. coli host strain XL1-Blue® (Stratagene). The high transformation efficiency of this bacterial strain increases the probability that the cDNA library will contain rare, under-represented clones. Alternative unidirectional vectors might include, but are not limited to, pcDNAI (Invitrogen, San Diego, CA) and pSHlox-1 (Novagen, Madison, WI).

**II Isolation of cDNA Clones**

The phagemid forms of individual cDNA clones were obtained by the in vivo excision process, in which XL1-BLUE was coinfected with an f1 helper

phage. Proteins derived from both lambda phage and f1 helper phage initiated new DNA synthesis from defined sequences on the lambda target DNA and create a smaller, single-stranded circular phagemid DNA molecule that includes all DNA sequences of the pBluescript plasmid and the cDNA insert. The phagemid DNA was released from the cells and purified, then used to reinfect fresh bacterial host cells (SOLR, Stratagene Inc), where the double-stranded phagemid DNA was produced. Because the phagemid carries the gene for ß-lactamase, the newly transformed bacteria were selected on medium containing ampicillin.

Phagemid DNA was purified using the QIAWELL-8 Plasmid Purification System from QIAGEN® DNA Purification System (QIAGEN Inc, Chatsworth, CA). This technique provides a rapid and reliable high-throughput method for lysing the bacterial cells and isolating highly purified phagemid DNA. The DNA eluted from the purification resin was suitable for DNA sequencing and other analytical manipulations.

An alternate method of purifying phagemid has recently become available. It utilizes the Miniprep Kit (Catalog No. 77468, Advanced Genetic Technologies Corporation, Gaithersburg, MD). This kit is in the 96-well format and provides enough reagents for 960 purifications. Each kit is provided with a recommended protocol, which has been employed except for the following changes. First, the 96 wells are each filled with only 1 ml of sterile terrific broth with carbenicillin at 25 mg/L and glycerol at 0.4%. After the wells are inoculated, the bacteria are cultured for 24 hours and lysed with 60 µl of lysis buffer. A centrifugation step (2900 rpm for 5 minutes) is performed before the contents of the block are added to the primary filter plate. The optional step of adding isopropanol to TRIS buffer is not routinely performed. After the last step in the protocol, samples are transferred to a Beckman 96-well block for storage.

**III Sequencing of cDNA Clones**

The cDNA inserts from random isolates of the pituitary library were sequenced by the method of Sanger F. and AR Coulson (1975; J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer) and reading frame determined.

**IV Homology Searching of cDNA Clones and Deduced Proteins**

Each sequence so obtained was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems Inc. and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (developed by TRW Inc., Los Angeles, CA) was used to

determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments of the protein sequence were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

Alternatively, BLAST, which stands for Basic Local Alignment Search Tool, was used to search for local sequence alignments (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10). BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologues. Although it is ideal for matches which do not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the high-scoring segment pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output. An E greater than or equal to 25 usually indicates that a match is significant.

The nucleotide sequence for the entire coding region (included within SEQ ID NO:1) of the human homolog of the mouse RAB18, HRAB18, is shown in Figure 1.

BLAST results showed that the coding sequence of the clone of the subject invention had an E parameter value of 156 when compared with that of the mouse rab18 gene (GenBank accession numbers X80333 and L04966). The coding sequence also shares HSP sequences with a human rab2 coding sequence (GenBank accession number M28213) with an E value of 54, and a human rab13 coding sequence (GenBank accession number X75593) with an E value of 51.

The coding sequence also shares HSP sequences with several clones in the LIFESEQ™ database (Incyte Pharmaceuticals, Palo Alto California) including Incyte clone 45334 derived from corneal stroma (E=37); Incyte clone 57291 derived from skeletal muscle (E=37); and Incyte clone 181288 derived from human placenta (E=36). All three tissue types are enervated. The presence of hrab18 related nucleotide sequences in these tissue types may result from the presence of nerve cells containing related RAB proteins involved in secretory vesicle trafficking. In addition, Incyte clone 269502 derived from a neuronal cell line (hNT) contains a coding sequence which shares high homology (87%) with the mouse rab18 gene.

**V Identification and Full Length Sequencing of the Genes**

The complete hrab18 nucleotide sequence was obtained from Incyte clone 112352. The sequence for the full length hrab18 gene was translated, and the putative in-frame translation is shown in Figure 1. When all three possible predicted translations of the sequence were searched against protein databases such as SwissProt and PIR, no exact matches were found to the possible translations of HRAB18. Figure 2 shows the comparison of the HRAB18 amino acid sequence with GenBank mouse RAB18. The substantial region of homology among these molecules encompasses the whole length of the molecule with only two out of 207 residues not conserved.

**VI Antisense analysis**

Knowledge of the cDNA sequence of the hrab18 gene will enable its use in antisense technology in the investigation of gene function. Oligonucleotides, genomic or cDNA fragments comprising the antisense strand of hrab18 are used either in vitro or in vivo to inhibit expression of the protein. Such technology is now well known in the art, and probes are designed at various locations along the nucleotide sequence. By transfection of cells or whole test animals with such antisense sequences, the gene of interest are effectively turned off. The function of the gene is ascertained by observing behavior at the cellular, tissue or organismal level (e.g. changes in secretory pathways, lethality, loss of differentiated function, changes in morphology, for example).

In addition to using sequences constructed to interrupt transcription of the open reading frame, modifications of gene expression are obtained by

designing antisense sequences to intron regions, promoter/enhancer elements, or even to trans-acting regulatory genes. Similarly, inhibition is achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing.

**VII Expression of HRAB18**

Expression of HRAB18 is accomplished by subcloning the cDNAs into appropriate expression vectors and transfecting the vectors into appropriate expression hosts. In this particular case, the cloning vector used in the generation of the full length clone also provides for expression of the included hrab18 sequence in E. coli. Upstream of the cloning site, this vector contains a promoter for ß-galactosidase, followed by sequence containing the amino-terminal Met and the subsequent 7 residues of ß-galactosidase. Immediately following these eight residues is an engineered bacteriophage promoter useful for artificial priming and transcription and a number of unique restriction sites, including Eco RI, for cloning.

Induction of the isolated, transfected bacterial strain with IPTG using standard methods will produce a fusion protein corresponding to the first seven residues of ß-galactosidase, about 15 residues of "linker", and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, it can be obtained by deletion or insertion of the appropriate number of bases by well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The hrab18 cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide amplimers containing cloning sites as well as a segment of DNA sufficient to hybridize to stretches at both ends of the target cDNA (25 bases) can be synthesized chemically by standard methods. These primers can then used to amplify the desired gene segments by PCR. The resulting new gene segments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments can be produced by digesting the cDNA with appropriate restriction enzymes and filling in the missing gene segments with chemically synthesized oligonucleotides. Segments of the coding sequence from more than one gene can be ligated together and cloned in appropriate vectors to optimize expression of the recombinant sequence.

Suitable expression hosts for such chimeric molecules include but are not limited to mammalian cells such as Chinese Hamster Ovary (CHO) and human

293 cells, insect cells such as Sf9 cells, yeast cells such as Saccharomyces cerevisiae, and bacteria such as E. coli. For each of these cell systems, a useful expression vector includes an origin of replication to allow propagation in bacteria and a selectable marker such as the ß-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors include a second selectable marker such as the neomycin phosphotransferase gene to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts require RNA processing elements such as 3' polyadenylation sequences if such are not part of the cDNA of interest.

Additionally, the vector may contain promoters or enhancers which increase gene expression. Such promoters are host specific and include MMTV, SV40, or metallothionine promoters for CHO cells; trp, lac, tac or T7 promoters for bacterial hosts, or alpha factor, alcohol oxidase or PGH promoters for yeast. Transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used in mammalian host cells. Once homogeneous cultures of recombinant cells are obtained through standard culture methods, large quantities of recombinantly produced HRAB18 can be recovered from the conditioned medium and analyzed using chromatographic methods known in the art.

**VIII Isolation of Recombinant HRAB18**

HRAB18 is expressed as a chimeric protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen) between the purification domain and the hrab18 sequence provides for purification of HRAB18 from the fusion protein.

**IX Production of HRAB18 Specific Antibodies**

Two approaches are utilized to raise antibodies to HRAB18, and each approach is useful for generating either polyclonal or monoclonal antibodies. In one approach, denatured protein from the reverse phase HPLC separation is obtained in quantities up to 75 mg. This denatured protein is used to immunize mice or rabbits using standard protocols; about 100 micrograms are adequate for immunization of a mouse, while up to 1 mg are used to immunize a rabbit. For identifying mouse hybridomas, the denatured protein is radioiodinated and used to screen potential murine B-cell hybridomas for

those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg is sufficient for labeling and screening several thousand clones.

In the second approach, the amino acid sequence of HRAB18, as deduced from translation of the cDNA, is analyzed to determine regions of high immunogenicity. Oligopeptides comprising appropriate hydrophilic regions, as shown in Figure 3, are synthesized and used in suitable immunization protocols to raise antibodies. Analysis to select appropriate epitopes is described by Ausubel FM et al., supra. The optimal amino acid sequences for immunization are at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the protein is in its natural conformation.

Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl-N- hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). If necessary, a cysteine is introduced at the N-terminus of the peptide to permit coupling to KLH. Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% BSA, reacting with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas are prepared and screened using standard techniques. Hybridomas of interest are detected by screening with labeled HRAB18 to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto, CA) are coated with affinity purified, specific rabbit-anti-mouse (or suitable anti-species Ig) antibodies at 10 mg/ml. The coated wells are blocked with 1% BSA, washed and exposed to supernatants from hybridomas. After incubation the wells are exposed to labeled HRAB18, 1 mg/ml. Clones producing antibodies will bind a quantity of labeled HRAB18 which is detectable above background. Such clones are expanded and subjected to 2 cycles of cloning at limiting dilution (1 cell/3 wells). Cloned hybridomas are injected into pristane mice to produce ascites, and monoclonal antibody is purified from mouse ascitic fluid by affinity chromatography on Protein A. Monoclonal antibodies with affinities of at least $10^8$ $M^{-1}$, preferably $10^9$ to $10^{10}$ or stronger, will be made by standard procedures as described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and in Goding (1986) Monoclonal

Antibodies: Principles and Practice, Academic Press, New York City, both incorporated herein by reference.

**X Diagnostic Test Using HRAB18 Specific Antibodies**

Particular HRAB18 antibodies are useful for the diagnosis of disorders which are characterized by differences in the amount or distribution of HRAB18 in the pituitary or other neuronally derived cells. Diagnostic tests for HRAB18 include methods utilizing the antibody and a label to detect HRAB18 in human bodily fluids, tissues or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, chromogenic agents, magnetic particles and the like. Patents teaching the use of such labels include US Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567, incorporated herein by reference.

A variety of protocols for measuring soluble or membrane-bound HRAB18, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HRAB18 is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211).

**XI Purification of Native HRAB18 Using Specific Antibodies**

Naturally occurring or recombinant HRAB18 is purified by immunoaffinity chromatography using antibodies specific for HRAB18. In general, an immunoaffinity column is constructed by covalently coupling the anti-HRAB18 antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, NJ). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia LKB Biotechnology). The antibody is

coupled to the resin, the resin is blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such immunoaffinity columns are utilized in the purification of HRAB18 by preparing a fraction from cells containing HRAB18 in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble HRAB18 containing a signal sequence is secreted in useful quantity into the medium in which the cells are grown.

A soluble HRAB18-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of RAB proteins (eg, high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/HRAB18 binding (e.g., a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and HRAB18 is collected.

**XII HRAB18 Localization and Activity**

HRAB18 may be localized in neuronal cells, particularly pituitary cells, in the following manner. First, either naturally-occurring HRAB18 or HRAB18 purified from E. Coli expressing the protein with its C-terminus prenylated in vitro is obtained. The prenylation allows HRAB18 to become localized to cellular compartments, such as the late endosomes, the trans Golgi network, the cis Golgi network, or the endoplasmic reticulum, within a cell. The prenylated HRAB18 is added to a cell-free system, such as one where the plasma membrane has been solubilized by digitonin. The HRAB18 is added at a concentration so as to observe specific binding of HRAB18 to specific cellular compartments. The localization is monitored with radiolabeled antibodies.

Once HRAB18 is localized to a specific cellular compartment, cell-free reconstitution studies may be performed to investigate its function. For example, a cell-free system can be
developed that is capable of measuring vesicular transport from the endoplasmic reticulum to the trans Golgi network. Preferably, this cell free system is depleted of naturally occurring HRAB18 to allow study of the effect of cell free systems lacking RAB18 on vesicular transport. The concentration of HRAB18 is gradually increased to recover HRAB18 activity in vesicular transport. This method is used to test HRAB18 derivatives for biological activity.

**XIII Drug Screening**

HRAB18 or host cells containing HRAB18 are used to screen compounds

that may affect vesicle trafficking by HRAB18, its isoforms or even other RAB proteins. The polypeptide or fragment employed in such a test is used in a cell free system or located intracellularly. One method of compound screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide of fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, alterations in vesicular transport of specific peptides or neurotransmitters.

Thus, the present invention provides methods of screening for test compounds which can affect HRAB18 activity. These methods comprise contacting a compound with HRAB18 and assaying for the presence of a complex between the compound and HRAB18 by methods well known in the art. After suitable incubation, free compound is separated from that in bound form, and the amount of bound compound is a measure of its ability to interfere in the regular functioning of HRAB18.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to HRAB18, and described in European Patent 84/03564, incorporated herein by reference.

Competitive drug screening assays in which neutralizing antibodies capable of binding HRAB18 specifically compete with a test compound for binding to HRAB18 are used to determine compounds which specifically bind HRAB18. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic determinants with HRAB18.

**XIV Rational Drug Design**

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact, including nonhydrolyzable analogs of GTP, for example. Any of these examples can be used to fashion drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide in vivo (Hodgson J (1991) Bio/Technology 9:19-21, incorporated herein by reference).

In one approach, the three-dimensional structure of a protein of interest, or of a protein-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide are ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide is gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous RAB-like molecules or to identify efficient inhibitors. Useful

examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton S and Wells JA (1992) Biochemistry 31:7796-7801 or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda SB et al (1993) J Biochem 113:742-746, incorporated herein by reference.

It is also possible to isolate a target-specific antibody, selected by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids is an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides act as the pharmacore.

HRAB18 is used to perform such analytical studies as X-ray crystallography. In addition, knowledge of the HRAB18 amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

**XV Use and Administration of Drugs**

Numerous diseases have been associated with the altered secretion of hormones or the abnormal recycling of surface receptors in secretory tissue, particularly pituitary tissue. For example, most cases of dwarfism are caused by a deficiency of all anterior pituitary secretion, while gigantism results from excessive activity and secretion of GH by somatropic cells. All these diseases may be associated with an abnormal regulation of vesicle transport, fusion and targeting associated with the altered expression of HRAB18. In addition, since HRAB18 may play a role in endocytosis, altered expression of HRAB18 may result in disorders related to the abnormal recycling of receptors. Since HRAB18 appears to regulate vesicular transport between intracellular compartments, compounds that bind HRAB18 may be used therapeutically to treat abnormal secretion of pituitary hormones or abnormal levels of receptors on the pituitary cell surface. Alternatively, these compounds may regulate the abnormal secretion of neurotransmitters from neuronal cells. Furthermore, HRAB18 itself may be administered to treat a disorder associated with the altered expression of HRAB18.

Therapeutic compounds are formulated in a nontoxic, inert, pharmaceutically acceptable aqueous carrier medium preferably at a pH of about 5 to 8, more preferably 6 to 8, although the pH may vary according to the characteristics of the formulation and its administration.

Characteristics such as solubility of the molecule, half-life and antigenicity/immunogenicity will aid in defining an effective carrier. Recombinant, organic or synthetic molecules resulting from drug design may be equally effective in particular situations.

Therapeutic compounds are delivered by known routes of administration including but not limited to topical creams and gels; transmucosal spray and aerosol, transdermal patch and bandage; injectable, intravenous and lavage formulations; and orally administered liquids and pills, particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration will be determined by the attending physician and will vary according to each specific situation.

Such determinations are made by considering multiple variables such as the condition to be treated, the therapeutic compound to be administered, and the pharmacokinetic profile of the particular therapeutic compound. Additional factors which may be taken into account include disease state (e.g. severity) of the patient, age, weight, gender, diet, time of administration, drug combination, reaction sensitivities, and tolerance/response to therapy. Long acting therapeutic compound formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular therapeutic HRAB18.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see US Patent Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different uses of therapeutic compounds and that administration targeting a tissue or organ may necessitate delivery in a specific manner.

All publications and patents mentioned in the above specification are herein incorporated by reference. The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above described modes for carrying out the invention which are readily apparent to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: INCYTE PHARMACEUTICALS, INC.

(ii) TITLE OF INVENTION: HUMAN HOMOLOG OF A MOUSE RAB 18 GENE

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: INCYTE PHARMACEUTICALS, INC.
(B) STREET: 3174 Porter Drive
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Glaister, Debra J.
(B) REGISTRATION NUMBER: 33888
(C) REFERENCE/DOCKET NUMBER: PF-0043 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415-855-0555
(B) TELEFAX: 415-845-4166

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(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1148 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Pituitary

(B) CLONE: 112352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGCACCCGGG CGGCCAGCTG GGCTCGGAGC GGAACGGGGT CAGGATGGAC GAGGACGTGC 60
TAACCACCCT GAAGATCCTC ATCATCGGCG AGAGTGGGGT GGGCAAGTCC AGCCTGCTCT 120

TGAGGTTCAC AGATGATACG TTTGATCCAG AACTTGCAGC AACAATAGGT GTTGACTTTA 180

AGGTGAAAAC AATTTCAGTG GATGGAAATA AGGCTAAACT TGCAATATGG GATACTGCTG 240

GTCAAGAGAG GTTTAGAACA TTAACTCCCA GCTATTATAG AGGTGCACAG GGTGTTATAT 300

TAGTTTATGA TGTCACAAGA AGAGATACAT TTGTTAAACT GGATAACTGG TTAAATGAAT 360

TGGAAACATA CTGTACAAGA AATGACATAG TAAACATGCT AGTTGGAAAT AAAATCGATA 420

AGGAAAATCG TGAAGTCGAT AGAAATGAAG GCCTGAAATT TGCACGAAAG CATTCCATGT 480

TATTTATAGA GGCAAGTGCA AAAACCTGTG ATGGTGTACA ATGTGCCTTT GAAGAACTTG 540

TTGAAAAGAT CATTCAGACC CCTGGACTGT GGGAAAGTGA GAACCAGAAT AAAGGAGTCA 600

AACTGTCACA CAGGGAAGAA GGCCAAGGAG GAGGAGCCTG TGGTGGTTAT TGCTCTGTGT 660

TATAAACTCT GGGAAATTCC ATCTCTTGCA TATTTGATCA GATAGTGACA TCTTTCTGTA 720

TATAAACTCT TTAACCTGCT ATTTTAGGGA CCTTGCAGTT TGCACATAAT TGTTTTATAT 780

CATAGCAGTA AATATTTGCA AGAAATCCCA CTCATCGACC CCGGGTAAAA TGTTATGGTA 840

AGCATGCACA GTTTGCAGTC TACAGTTTTT TTATGTAGCA CCAAATAGGT GTACCTTTAT 900

AAGTACATTC AATTTTATGA TTTACATTTA TCATGTAATT TTTAAAAAAA TCCATCTATC 960

TAGGATATGT TGATACAAAG TCTGCTTTTG CTATTCTTTT TGCTTAAATA CTCCTATCAT 1020

TTTCTGAATT ACTTGGTATT TAGAACTCCT AGCACCACGG GGAAGAATAG AGGTATCATC 1080

AAACGTGGCA AATTTTCTTT CAGGAATAAT AAAGAGCATG ATTCCACAGC CAAAAAAAAA 1140

AAAAAAAA 1148

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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1 5 10 15

Ser Gly Val Gly Lys Ser Ser Leu Leu Leu Arg Phe Thr Asp Asp Thr
20 25 30

```
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          35                  40                  45

Thr Ile Ser Val Asp Gly Asn Lys Ala Lys Leu Ala Ile Trp Asp Thr
      50                  55                  60

Ala Gly Gln Glu Arg Phe Arg Thr Leu Thr Pro Ser Tyr Tyr Arg Gly
 65                  70                  75                  80

Ala Gln Gly Val Ile Leu Val Tyr Asp Val Thr Arg Arg Asp Thr Phe
                 85                  90                  95

Val Lys Leu Asp Asn Trp Leu Asn Glu Leu Glu Thr Tyr Cys Thr Arg
            100                 105                 110

Asn Asp Ile Val Asn Met Leu Val Gly Asn Lys Ile Asp Lys Glu Asn
        115                 120                 125

Arg Glu Val Asp Arg Asn Glu Gly Leu Lys Phe Ala Arg Lys His Ser
    130                 135                 140

Met Leu Phe Ile Glu Ala Ser Ala Lys Thr Cys Asp Gly Val Gln Cys
145                 150                 155                 160

Ala Phe Glu Glu Leu Val Glu Lys Ile Ile Gln Thr Pro Gly Leu Trp
                165                 170                 175

Glu Ser Glu Asn Gln Asn Lys Gly Val Lys Leu Ser His Arg Glu Glu
            180                 185                 190

Gly Gln Gly Gly Gly Ala Cys Gly Gly Tyr Cys Ser Val Leu
        195                 200                 205
```

CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide having the sequence as depicted in SEQ ID NO:2, or its complement.

2. The polynucleotide of Claim 1 wherein the nucleic acid sequence comprises the sequence shown in SEQ ID NO:1 from nucleotide 45 to nucleotide 664.

3. A purified polynucleotide comprising the nucleic acid sequence of SEQ ID NO:1 from nucleotide 1 to nucleotide 44.

4. A purified polynucleotide comprising the nucleic acid sequence of SEQ ID NO:1 from nucleotide 665 to 1148.

5. An expression vector comprising the polynucleotide of Claim 1.

6. A host cell comprising the expression vector of Claim 5.

7. An antisense molecule comprising a polynucleotide sequence complementary to at least a portion of the polynucleotide of Claim 2.

8. A method for producing a polypeptide comprising the sequence as depicted in SEQ ID NO:2, said method comprising:

a) culturing the host cells of Claim 6 under conditions suitable for the expression of the polypeptide, and

b) recovering said polypeptide from the cell culture.

9. Purified HRAB18 having the amino acid sequence as depicted in SEQ ID NO:2.

10. An antibody specific for the purified polypeptide of Claim 9.

11. A method of screening a plurality of test compounds for binding to the polypeptide of Claim 9, or a portion thereof, said method comprising the steps of:

a) providing a plurality of test compounds;

b) combining the polypeptide of Claim 9, or a portion thereof, with each of the test compounds for a time sufficient to allow binding under

suitable conditions; and

c) detecting binding of the polypeptide of Claim 9 or a fragment thereof, to each of the plurality of compounds, thereby identifying the compounds which specifically bind the polypeptide of Clain 9 or a fragment thereof.

12. A diagnostic test for the detection of nucleic acid sequences encoding HRAB18 in a biological sample, comprising the steps of:

a) combining the biological sample with a polynucleotide which comprises the nucleic acid sequence of SEQ ID NO:1, cr a fragment thereof, under conditions suitable for the formation of a nucleic acid hybridization complex between the nucleic acid sequence of SEQ ID NO:1 and a complementary nucleic acid sequence in said sample,

b) detecting said hybridization complex, and

c) comparing the amount of said hybridization complex with a standard wherein the presence of an abnormal level of said hybridization complex correlates positively with a condition associated with altered expression of HRAB18.

13. A diagnostic test for the detection of nucleotide sequences encoding HRAB18 in a biological sample, comprising the steps of:

a)combining the biological sample with polymerase chain reaction primers under conditions suitable for nucleic acid amplification, wherein said primers comprise fragments from the nucleotide sequence of SEQ ID NO:1;

b) detecting amplified nucleotide sequences; and

c) comparing the amount of amplified nucleotide sequences in said biological sample with a standard thereby determining whether the amount of said nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said nucleotide sequence correlates positively with a condition associated with altered expression of HRAB18..

```
ATG GAC GAG GAC GTG CTA ACC ACC CTG AAG ATC CTC ATC ATC GGC GAG          48
Met Asp Glu Asp Val Leu Thr Thr Leu Lys Ile Leu Ile Ile Gly Glu
 1               5                  10                  15

AGT GGG GTG GGC AAG TCC AGC CTG CTC TTG AGG TTC ACA GAT GAT ACG          96
Ser Gly Val Gly Lys Ser Ser Leu Leu Leu Arg Phe Thr Asp Asp Thr
            20                  25                  30

TTT GAT CCA GAA CTT GCA GCA ACA ATA GGT GTT GAC TTT AAG GTG AAA         144
Phe Asp Pro Glu Leu Ala Ala Thr Ile Gly Val Asp Phe Lys Val Lys
        35                  40                  45

ACA ATT TCA GTG GAT GGA AAT AAG GCT AAA CTT GCA ATA TGG GAT ACT         192
Thr Ile Ser Val Asp Gly Asn Lys Ala Lys Leu Ala Ile Trp Asp Thr
    50                  55                  60

GCT GGT CAA GAG AGG TTT AGA ACA TTA ACT CCC AGC TAT TAT AGA GGT         240
Ala Gly Gln Glu Arg Phe Arg Thr Leu Thr Pro Ser Tyr Tyr Arg Gly
65                  70                  75                  80

GCA CAG GGT GTT ATA TTA GTT TAT GAT GTC ACA AGA AGA GAT ACA TTT         288
Ala Gln Gly Val Ile Leu Val Tyr Asp Val Thr Arg Arg Asp Thr Phe
                85                  90                  95

GTT AAA CTG GAT AAC TGG TTA AAT GAA TTG GAA ACA TAC TGT ACA AGA         336
Val Lys Leu Asp Asn Trp Leu Asn Glu Leu Glu Thr Tyr Cys Thr Arg
            100                 105                 110

AAT GAC ATA GTA AAC ATG CTA GTT GGA AAT AAA ATC GAT AAG GAA AAT         384
Asn Asp Ile Val Asn Met Leu Val Gly Asn Lys Ile Asp Lys Glu Asn
        115                 120                 125

CGT GAA GTC GAT AGA AAT GAA GGC CTG AAA TTT GCA CGA AAG CAT TCC         432
Arg Glu Val Asp Arg Asn Glu Gly Leu Lys Phe Ala Arg Lys His Ser
    130                 135                 140

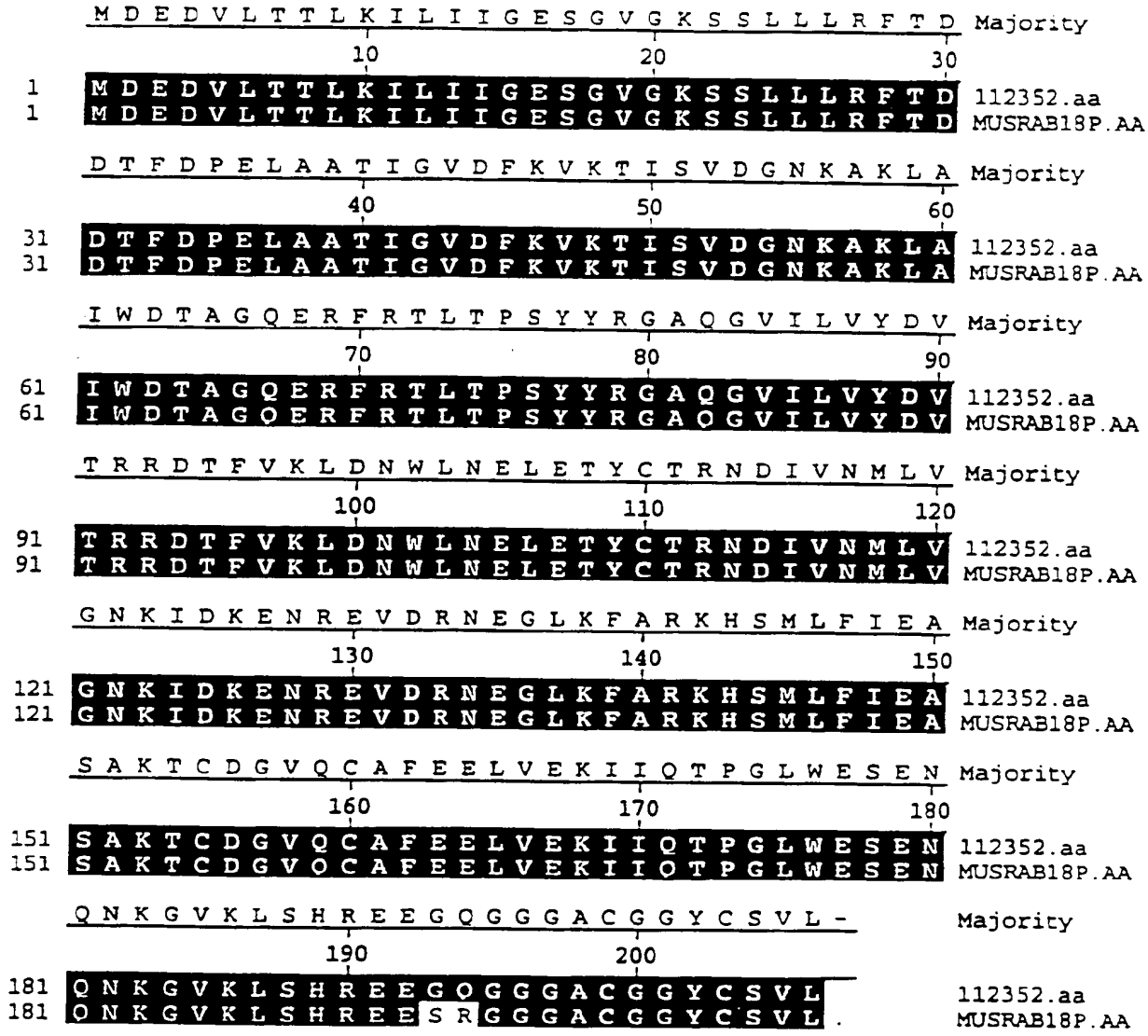
ATG TTA TTT ATA GAG GCA AGT GCA AAA ACC TGT GAT GGT GTA CAA TGT         480
Met Leu Phe Ile Glu Ala Ser Ala Lys Thr Cys Asp Gly Val Gln Cys
145                 150                 155                 160

GCC TTT GAA GAA CTT GTT GAA AAG ATC ATT CAG ACC CCT GGA CTG TGG         528
Ala Phe Glu Glu Leu Val Glu Lys Ile Ile Gln Thr Pro Gly Leu Trp
                165                 170                 175

GAA AGT GAG AAC CAG AAT AAA GGA GTC AAA CTG TCA CAC AGG GAA GAA         576
Glu Ser Glu Asn Gln Asn Lys Gly Val Lys Leu Ser His Arg Glu Glu
            180                 185                 190

GGC CAA GGA GGA GGA GCC TGT GGT GGT TAT TGC TCT GTG TTA TAA             621
Gly Gln Gly Gly Gly Ala Cys Gly Gly Tyr Cys Ser Val Leu  *
        195                 200                 205
```

# FIGURE 1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181 QNKGVKLSHREESRGGGACGGYCSVL. MUSRAB18P.AA

**FIGURE 2**

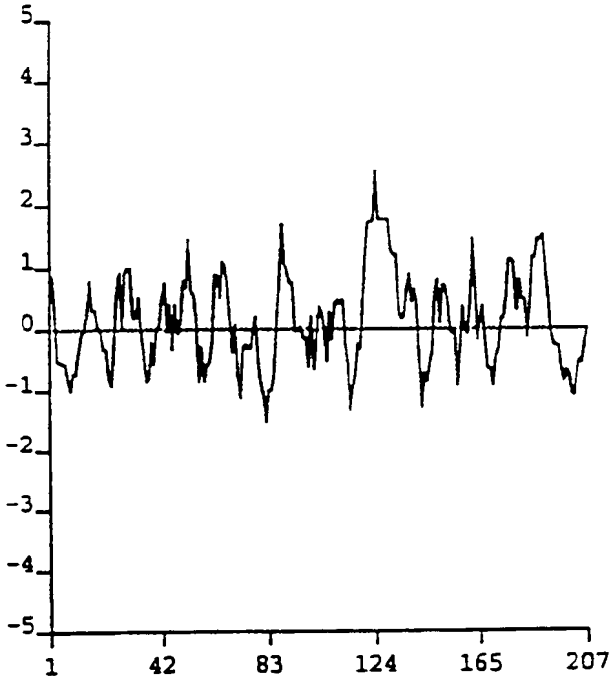


**FIGURE 3**

# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/10699

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C07K16/18 C12Q1/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | GENE, vol. 132, no. 2, 15 October 1993, AMSTERDAM NL, pages 273-278, XP002015898 HELEN YU ET AL.: "Gene cloning and characterization of a GTP-binding Rab protein from mouse pituitary AtT-20 cells" cited in the application see abstract see page 274, left-hand column, paragraph 2 - page 278, left-hand column, line 1; figure 2 --- -/-- | 1-13 |

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 15 October 1996 | 25. 10. 96 |

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Inter nal Application No
PCT/US 96/10699

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | JOURNAL OF CELL SCIENCE, vol. 107, no. 12, December 1994, pages 3437-3448, XP000604112 ANNE LÜTCKE ET AL.: "Cloning and subcellular localization of a novel rab proteins reveals polarized and cell type-specific expression" see page 3438, left-hand column, paragraph 6 - right-hand column, paragraph 1 see page 3439, right-hand column, paragraph 1 - paragraph 2; figure 1 --- | 1-13 |
| A | JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 21, 25 July 1989, MD US, pages 12394-12401, XP002015899 A. ZAHRAOUI ET AL.: "The human Rab genes encode a family of GTP-binding proteins related to yeast YPT1 and SEC4 products involved in secretion" --- | 1-13 |
| A | EMBL Database Entry HS79235; Accession number T64792; 6 March 1995; HILLIER L. ET AL.: "The WashU-Merck EST Project" XP002015900 ----- | 1-4 |

1

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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**A1**

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(30) Priority Data:

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|---|---|---|
| 60/000,377 | 21 June 1995 (21.06.95) | US |
| 08/569,062 | 6 December 1995 (06.12.95) | US |


(71) Applicant: INCYTE PHARMACEUTICALS, INC. [US/US]; 3174 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors: KENNEDY, Neil, F.; 6571 Jeremie Drive, San Jose, CA 95120 (US). GUEGLER, Karl, J.; 1048 Oakland Avenue, Menlo Park, CA 94025 (US). SEILHAMER, Jeffrey, J.; 12555 La Cresta, Los Altos Hills, CA 94022 (US).

(74) Agent: GLAISTER, Debra, J.; Incyte Pharmaceuticals, Inc., 3174 Porter Drive, Palo Alto, CA 94304 (US).

(81) Designated States: AT, AU, BR, CA, CH, CN, DE, DK, ES, FI, GB, IL, JP, KR, MX, NO, NZ, RU, SE, SG, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

**Published**
*With international search report.*

(54) Title: HUMAN HOMOLOG OF THE MOUSE RAB18 GENE

(57) Abstract

The present invention provides nucleotide and amino acid sequences that identify and encode a human homolog of mouse RAB18 (HRAB18) expressed in human pituitary. The present invention also provides for antisense molecules to the nucleotide sequences which encode HRAB18, hybridization probes or oligonucleotides for the detection of HRAB18-encoding nucleotide sequences, and a diagnostic test based on HRAB18-encoding nucleic acid molecules. The present invention further provides for genetically engineered host cells for the expression of HRAB18, biologically active HRAB18, antibodies capable for binding specifically to HRAB18, and treatment methods comprising administration of compounds capable of binding HRAB18.

```
ATG GAC GAG GAC GTG CTA ACC ACC CTG AAG ATC CTC ATC ATC GGC GAG          48
Met Asp Glu Asp Val Leu Thr Thr Leu Lys Ile Leu Ile Ile Gly Glu
 1               5                  10                  15

AGT GGG GTG GGC AAG TCC AGC CTG CTC TTG AGG TTC ACA GAT GAT ACG          96
Ser Gly Val Gly Lys Ser Ser Leu Leu Leu Arg Phe Thr Asp Asp Thr
            20                  25                  30

TTT GAT CCA GAA CTT GCA GCA ACA ATA GGT GTT GAC TTT AAG GTG AAA         144
Phe Asp Pro Glu Leu Ala Ala Thr Ile Gly Val Asp Phe Lys Val Lys
        35                  40                  45

ACA ATT TCA GTG GAT GGA AAT AAG GCT AAA CTT GCA ATA TGG GAT ACT         192
Thr Ile Ser Val Asp Gly Asn Lys Ala Lys Leu Ala Ile Trp Asp Thr
    50                  55                  60

GCT GGT CAA GAG AGG TTT AGA ACA TTA ACT CCC AGC TAT TAT AGA GGT         240
Ala Gly Gln Glu Arg Phe Arg Thr Leu Thr Pro Ser Tyr Tyr Arg Gly
65                  70                  75                  80

GCA CAG GGT GTT ATA TTA GTT TAT GAT GTC ACA AGA AGA GAT ACA TTT         288
Ala Gln Gly Val Ile Leu Val Tyr Asp Val Thr Arg Arg Asp Thr Phe
                85                  90                  95

GTT AAA CTG GAT AAC TGG TTA AAT GAA TTG GAA ACA TAC TGT ACA AGA         336
Val Lys Leu Asp Asn Trp Leu Asn Glu Leu Glu Thr Tyr Cys Thr Arg
            100                 105                 110

AAT GAC ATA GTA AAC ATG CTA GTT GGA AAT AAA ATC GAT AAG GAA AAT         384
Asn Asp Ile Val Asn Met Leu Val Gly Asn Lys Ile Asp Lys Glu Asn
        115                 120                 125

CGT GAA GTC GAT AGA AAT GAA GGC CTG AAA TTT GCA CGA AAG CAT TCC         432
Arg Glu Val Asp Arg Asn Glu Gly Leu Lys Phe Ala Arg Lys His Ser
    130                 135                 140

ATG TTA TTT ATA GAG GCA AGT GCA AAA ACC TGT GAT GGT GTA CAA TGT         480
Met Leu Phe Ile Glu Ala Ser Ala Lys Thr Cys Asp Gly Val Gln Cys
145                 150                 155                 160

GCC TTT GAA GAA CTT GTT GAA AAG ATC ATT CAG ACC CCT GGA CTG TGG         528
Ala Phe Glu Glu Leu Val Glu Lys Ile Ile Gln Thr Pro Gly Leu Trp
                165                 170                 175

GAA AGT GAG AAC CAG AAT AAA GGA GTC AAA CTG TCA CAC AGG GAA GAA         576
Glu Ser Glu Asn Gln Asn Lys Gly Val Lys Leu Ser His Arg Glu Glu
            180                 185                 190

GGC CAA GGA GGA GGA GCC TGT GGT GGT TAT TGC TCT GTG TTA TAA             621
Gly Gln Gly Gly Gly Ala Cys Gly Gly Tyr Cys Ser Val Leu  *
        195                 200                 205
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| **FI** | Finland | **ML** | Mali | **US** | United States of America |
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| **GA** | Gabon | **MR** | Mauritania | **VN** | Viet Nam |

# HUMAN HOMOLOG OF MOUSE RAB18 GENE

## TECHNICAL FIELD

The present invention is in the field of molecular biology; more particularly, the present invention describes the nucleic acid and amino acid sequences of a human homolog of the mouse rab18 gene.

## BACKGROUND ART

RAB proteins belong to the RAS superfamily of G proteins that comprises nearly 50 related monomeric GTPases with molecular weights between about 20,000 to 30,000. Monomeric G proteins may interact with several types of effector proteins to trigger specific cellular responses. RAB proteins act as specific regulators of intracellular membrane trafficking, exocytosis, and endocytosis to control vesicle budding, targeting and fusion. RAS proteins activate a cascade of serine/threonine protein kinases to regulate cell growth and differentiation. RHO and RAC proteins are involved in relaying signals from cell-surface receptors to the actin cytoskeleton. (Alberts, B et al. Molecular Biology of the Cell, 3rd ed, Garland Publishing, Inc., New York City , NY (1994)).

G proteins exist in equilibrium between two forms, a GTP-bound form which is active and interacts with effector proteins, and a GDP-bound form which is inactive. The distribution of active and inactive G proteins appears to be modulated in part by certain regulatory proteins that affect the rates of GDP release or GTP hydrolysis by G proteins. For example, guanine nucleotide release proteins (GNRPs) catalyze the release of bound GDP. Subsequently GTP binds to the nucleotide binding site, and the G protein is activated. Alternatively, GTPase-activating proteins (GAPs) increase the rate of hydrolysis of GTP with concomitant production of GDP and phosphate. The GDP remains bound to the G protein and inactivates the protein. Other G proteins interact with a guanine nucleotide dissociation inhibitor (GDI) that inhibits the release of GDP (Barangar (1994) J Biol Chem 269:13637-43).

Most information on monomeric G proteins has been obtained by studying the structure and function of RAS proteins as described in Hesketh R, The Oncogene FactsBook, Academic Press, Great Britain (1995). Much less is known about the structural features important for the activity of other members of the RAS superfamily.

### RAB Proteins

So far, over 30 RAB proteins have been identified in mammalian cells with sequences that share between 35% and 95% identity indicating a broad range of functional specificities. Usually, these proteins have been localized to specific organelles. For example, RAB1 is localized to the ER and Golgi complex, RAB2 in the transitional ER and the cis Golgi network, RAB3 to secretory vesicles, RAB4 to early endosomes, RAB5 to early endosomes and the plasma membrane, RAB6 to medial and trans Golgi

cisternae, RAB7 to late endosomes, and RAB9 to late endosomes and the trans Golgi network (Alberts, supra).

In addition, RAB proteins are localized to specific tissue types. For example, RAB17 is found in epithelial cells which contains distinct apical, basolateral, and transcytotic transport pathways. Isoforms of RAB3 with about 77-85% homology appear to be largely restricted to cell lineages containing regulated secretory pathways, such as neurons, endocrine, and exocrine cells (Fischer von Mollard (1994) J Biol Chem 269: 10971-74). RAB18 is found to be expressed at a high level in the mouse brain, at a moderate level in the pituitary gland, and at low levels in the liver. This protein may play a role in secretory vesicle recycling (Yu H et al (1993) Gene 132:273-8).

Both RAB and RAS proteins appear to share conserved domains which are involved in guanine nucleotide binding or are involved in the conformational changes associated with GTP binding and GTP hydrolysis. Characteristic structural motifs associated with the GTP binding site include a first motif, GX$_4$GK(S/T), which interacts with the alpha and beta phosphates of GDP or GTP. Another motif, DXXG, also appears to interact with the gamma phosphate. A third motif, (N/T)(K/Q)XD, interacts with the guanine ring. A tightly bound $Mg^+$ is coordinated to a conserved threonine residue and to the beta and gamma phosphate groups of GTP. In addition the $Mg^+$ interacts with the serine/threonine residue of the first motif, and with the invariant aspartate of the third motif. Domains that appear important for conformational changes include the effector L2 loop and the helix a2/loop5 (a2L5) which appear to be involved in interactions with specific GEPs and GAPs (Ferro-Novick S. (1993) Ann. Rev. Cell Biol. 9:575-99).

In addition, posttranslational modification by a lipid moiety is critical for membrane localization and the proper activity of RAB. This modification occurs at the C-terminal end of the RAB proteins whereby a geranylgeranyl (GG) moiety, a 20-carbon isoprene unit, is usually attached via a thioether bond to one of two cysteine residues. Most RAB proteins have C termini that end in -XXCC (35%), -XCXC (37%), -CCXX (15%), -CCXXX (8%) and -CXXX (5%). Some RAB proteins, such as RAB3A, that have the -XCXC motif appear to be geranylgeranylated on each of the adjacent cysteine residues. (Farnsworth (1994) Proc Natl Acad Sci USA 91: 11963-7). This modification reaction appears to involve a single RAB-specific geranylgeranyltransferase (RAB GGTase II) that transfers the lipid moieties to the different RAB motifs. A RAB escort protein (REP) additionally participates in the lipidation reaction by binding the protein substrate, and then by forming a complex with RAB GGTase II. Then, the GGTase II transfers the geranylgeranyl moiety from geranylgeranylpyrophosphate to the protein substrate.

RAB proteins' mode of action as regulators of membrane trafficking

between intracellular compartments is not well understood. It has been proposed that RAB proteins cycle between soluble and membrane-bound forms and interact with vesicular and target membrane-bound proteins. When a RAB protein is bound by GDP (i.e., in an inactive form), it exists in a conformation in which its lipid moiety is hidden within the protein. Therefore, the protein remains in soluble form. Once the RAB protein is activated by a GNRP, the GDP is exchanged for GTP which alters the conformation of the protein so that the lipid moiety remains exposed and RAB becomes membrane-bound.

Membrane-bound RAB with GTP at the nucleotide binding site is localized where membrane vesicles are being pinched off and binds with a complex of certain vesicle specific proteins (v-SNARE). The RAB protein remains bound to the vesicle surface until the vesicle docks at the target membrane at which time the v-SNARE interacts with target associated SNARE (t-SNARE). At this time the GTP bound to RAB is hydrolyzed to GDP. Concomitantly, RAB alters its conformation so that its lipid moiety no longer is exposed and RAB is released from the target-membrane surface. Therefore, it appears that SNARE complexes may serve as the ultimate targets of regulation by RAB (Alberts, supra).

## DISCLOSURE OF THE INVENTION

The present invention relates to polynucleotides and polypeptides of a human homolog of mouse RAB18 designated herein as HRAB18. The present invention also provides for HRAB18 antisense DNA and expression vectors and host cells comprising polynucleotides encoding HRAB18.

Furthermore, the subject invention provides a method for producing HRAB18, and a purified HRAB18 polypeptide having the sequence shown in SEQ ID NO:2.

The subject invention also relates to diagnostic tests and compositions for the detection of disorders associated with altered expression of HRAB18, and more particularly, disorders associated with the pituitary gland.

A method of screening a plurality of test compounds to identify compounds binding to HRAB18 is also proposed along with their use as therapeutic compounds for the treatment of disorders related to the altered expression of HRAB18.

## BRIEF DESCRIPTION OF DRAWINGS

Figure 1 displays the nucleotide sequence (SEQ ID NO:1) and predicted amino acid sequence (SEQ ID NO:2) for HRAB18 found in Incyte clone 112352.

Figure 2 shows the amino acid alignment of HRAB18 with mouse RAB18. Alignments shown were produced using the multisequence alignment program of DNASTAR software (DNASTAR Inc, Madison WI).

Figure 3 shows a hydrophobicity plot for the amino acid sequence of HRAB18 using the hydrophobicity program of DNASTAR.

## MODES FOR CARRYING OUT THE INVENTION

**Definitions**

As used herein, the term "human homolog of mouse rab18" or "HRAB18" refers to the polypeptide as shown in SEQ ID NO:2. Polynucleotide sequences encoding HRAb18 were found in a human pituitary cDNA library. HRAB18 is a member of the RAB subfamily of monomeric G proteins and may be involved in the regulation of secretory vesicle recycling. In one embodiment disclosed herein, HRAB18 is encoded by the polynucleotide shown in SEQ ID NO:1 beginning with nucleotide 45 and ending with nucleotide 664. The present invention also relates to the upstream and downstream sequences shown in SEQ ID NO:1, that is, nucleotides 1 to 44 and 665 to 1148 in SEQ ID NO:1 which may affect mRNA transcript stability. HRAB18 may be naturally occurring, recombinantly produced or chemically synthesized. Also included within the scope of the present invention are active fragments of HRAB18. As used herein, the lower case "hrab18" refers to a nucleic acid sequence while the upper case "HRAB18" refers to a protein, peptide or amino acid sequence.

"Active" refers to those forms of HRAB18 which retain the biologic and/or immunologic activities of naturally occurring HRAB18.

"Naturally occurring HRAB18" refers to HRAB18 produced by human cells that have not been genetically engineered and specifically contemplates various HRAB18 forms arising from post-translational modifications of the polypeptide including but not limited to acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

"Derivative" refers to polypeptides derived from naturally occurring HRAB18 by chemical modifications such as ubiquitination, labeling (e.g., with radionuclides, various enzymes, etc.), pegylation (derivatization with polyethylene glycol) or by insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

"Recombinant variant" refers to any polypeptide differing from naturally occurring HRAB18 by amino acid insertions, deletions, and substitutions, created using recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest may be found by comparing the sequence of the particular HRAB18 with that of other RAB proteins and minimizing the number of amino acid sequence changes made in regions of high homology.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, or a threonine with a serine, i.e., conservative amino acid replacements. "Insertions" or

"deletions" are typically in the range of about 1 to 5 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in HRAB18 using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Where desired, a "signal or leader sequence" can direct the HRAB18 polypeptide through the membrane of a cell. Such a sequence may be naturally present on the HRAB18 polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, often at least about 7 amino acids, typically at least about 9 to 13 amino acids, and, in various embodiments, at least about 17 or more amino acids. To be active, HRAB18 polypeptides must have sufficient length to display biologic and/or immunologic activity.

An "oligonucleotide" or polynucleotide "fragment", "portion," or "segment" is a stretch of nucleotide residues which is long enough to use in polymerase chain reaction (PCR) or various hybridization procedures to identify or amplify HRAB18 mRNA or DNA molecules.

The present invention includes purified HRAB18 polypeptides from natural or recombinant sources, ie, cells transformed with recombinant nucleic acid molecules encoding HRAB18. Various methods for the isolation of the HRAB18 polypeptides may be accomplished by procedures well known in the art. For example, such polypeptides may be purified by immunoaffinity chromatography by employing the antibodies provided by the present invention. Various other methods of protein purification well known in the art include those described in Deutscher M (1990) Methods in Enzymology, Vol 182, Academic Press, San Diego; and Scopes R (1982) Protein Purification: Principles and Practice. Springer-Verlag, NYC, both incorporated herein by reference.

"Recombinant" refers to a polynucleotide which encodes HRAB18 and is prepared using recombinant DNA techniques. The DNA which encodes HRAB18 may also include allelic or recombinant variants and mutants thereof.

"Oligonucleotides" or "nucleic acid probes" are prepared based on the cDNA sequence which encodes HRAB18 (SEQ ID NO:2). Oligonucleotides comprise portions of the DNA sequence having between 10 and 60 nucleotides and preferably between 15 nucleotides and 60 nucleotides. Nucleic acid probes comprise portions of the sequence having fewer nucleotides than about 6 kb, usually fewer than about 1 kb. In one embodiment of the present invention, the oligonucleotide probes will comprise sequence that is identical or complementary to a portion of HRAB18 where there is little or no identity or complementarity with any known or prior art molecule. After appropriate testing to eliminate false positives, these probes may be

used to determine whether mRNA encoding HRAB18 is present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh PS et al (1992 PCR Methods Appl 1:241-250).

Probes may be derived from naturally occurring or recombinant single- or double-stranded nucleic acids or be chemically synthesized. They may be labeled by nick translation, Klenow fill-in reaction, PCR or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook J et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel FM et al (1989) Current Protocols in Molecular Biology, John Wiley & Sons, NYC, both incorporated herein by reference.

Alternatively, recombinant variants encoding HRAB18 may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations may also be introduced to modify the properties of the polypeptide, to change ligand-binding affinities, interchain affinities, or polypeptide degradation or turnover rate.

The present invention, in one aspect, provides a nucleotide sequence identified in Incyte 112352 encoding HRAB18, a human homolog of mouse rab18 gene. In another aspect, the present invention provides purified HRAB18 polypeptide from natural or recombinant sources. The amino acid sequence is shown in SEQ ID NO:2.

One embodiment of the subject invention is to provide for hrab18-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences encoding HRAB18. Further embodiments of the present invention are cells transformed with recombinant nucleic acid molecules encoding HRAB18 and antibodies to HRAB18.

Polynucleotides, polypeptides and antibodies to HRAB18 may be useful in diagnostic assays for detection of disorders of the regulation of intermembrane trafficking, such as, for example, endocytosis or exocytosis and as diagnostic compositions for the detection of disorders of secretory tissue, particularly neuronal and pituitary tissue. Additionally, these diagnostic tools may be useful in diagnosing disorders associated with tissue damage.

The nucleotide sequence encoding HRAB18 has numerous applications in techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use in the construction of oligomers for PCR, use for chromosome and gene mapping, use in the recombinant production of HRAB18, and use in generation of anti-sense DNA or RNA, their chemical analogs and the like. Uses of nucleotides encoding HRAB18 disclosed herein are exemplary of known techniques and are not

intended to limit their use in any technique known to a person of ordinary skill in the art. Furthermore, the nucleotide sequences disclosed herein may be used in molecular biology techniques that have not yet been developed, provided the new techniques rely on properties of nucleotide sequences that are currently known (eg, the triplet genetic code, and specific base pair interactions).

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of HRAB18-encoding nucleotide sequences, some bearing minimal homology to the nucleotide sequence of any known and naturally occurring gene may be produced. The invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the nucleotide sequence of naturally occurring HRAB18, and all such variations are to be considered as being specifically disclosed.

Although the nucleotide sequences which encode HRAB18 and/or its variants are preferably capable of hybridizing to the nucleotide sequence of naturally occurring HRAB18 under stringent conditions, it may be advantageous to produce nucleotide sequences encoding HRAB18 or its derivatives possessing a substantially different codon usage. Codons can be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic expression host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding HRAB18 and/or its derivatives without altering the encoded amino acid sequence include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

Nucleotide sequences encoding HRAB18 may be joined to a variety of other nucleotide sequences by means of well established recombinant DNA techniques (Sambrook J et al. supra). Useful nucleotide sequences for joining to hrab18 include an assortment of cloning vectors, e.g., plasmids, cosmics, lambda phage derivatives, phagemids, and the like, that are well known in the art. Vectors of interest include expression vectors, replication vectors, probe generation vectors, sequencing vectors, and the like. In general, vectors of interest may contain an origin of replication functional in at least one organism, convenient restriction endonuclease sensitive sites, and selectable markers for the host cell.

The subject invention provides for hrab18-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences encoding HRAB18. Such probes may also be used for the detection of other rab gene encoding sequences and should preferably

contain at least 50% of the nucleotides from the conserved region or active site. The hybridization probes of the subject invention may be derived from the nucleotide sequences of the SEQ ID NO:1 or from genomic sequences including promoters, enhancers and/or possible introns of respective naturally occurring hrab18 polynucleotides. Hybridization probes may be labeled by a variety of reporter groups, including radionuclides such as $^{32}P$ or $^{35}S$, or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

In addition, the subject invention provides for nucleic hybridization probes capable of hybridizing with either upstream or downstream sequences that may play a role in HRAB18 translation. Such probes may also be used to detect similar regulatory sequences for polypeptide translation.

PCR, as described US Patent Nos. 4,683,195; 4,800,195; and 4,965,188, provides additional uses for oligonucleotides based upon the nucleotide sequence which encodes HRAB18. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both and comprise a discrete nucleotide sequence for diagnostic use or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Full length genes may be cloned from known sequence using a new method which employs XL-PCR (Perkin-Elmer, Foster City, CA) to amplify long pieces of DNA. This method was developed to allow a single researcher to process multiple genes (up to 20 or more) at a time and to obtain an extended (possibly full-length) sequence within 6-10 days. It replaces current methods which use labelled probes to screen libraries and allow one researcher to process only about 3-5 genes in 14-40 days.

In the first step, which can be performed in about two days, primers are designed and synthesized based on a known partial sequence. In step 2, which takes about six to eight hours, the sequence is extended by PCR amplification of a selected library. Steps 3 and 4, which take about one day, are purification of the amplified cDNA and its ligation into an appropriate vector. Step 5, which takes about one day, involves transforming and growing up host bacteria. In step 6, which takes approximately five hours, PCR is used to screen bacterial clones for extended sequence. The final steps, which take about one day, involve the preparation and sequencing of selected clones. If the full length cDNA has not been obtained, the entire procedure is repeated using either the original library or some other preferred library.

The preferred library may be one that has been size-selected to include only larger cDNAs or may consist of single or combined commercially available libraries, eg. lung, liver, heart and brain from Gibco/BRL (Gaithersburg MD). The cDNA library may have been prepared with oligo dT or random primers. The advantage of using random primed libraries is that

generally have more sequences which contain 5' ends of genes. A randomly primed library may be particularly useful if an oligo dT library does not yield a complete gene. Obviously, the larger the protein, the less likely it is that the complete gene will be found in a single plasmid.

Other means of producing specific hybridization probes for hrab18 DNAs include the cloning of nucleic acid sequences encoding HRAB18 or HRAB18 derivatives into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides.

It is now possible to produce a DNA sequence, or portions thereof, encoding HRAB18 and their derivatives entirely by synthetic chemistry, after which the gene can be inserted into any of the many available DNA vectors using reagents, vectors and cells that are known in the art at the time of the filing of this application. Moreover, synthetic chemistry may be used to introduce mutations into the hrab18 sequences or any portion thereof. The nucleotide sequence of hrab18 sequences can be confirmed through DNA sequencing techniques.

Methods for DNA sequencing are well known in the art. Conventional enzymatic methods employed DNA polymerase Klenow fragment, SEQUENASE® (US Biochemical Corp, Cleveland, OH) or Taq polymerase to extend DNA chains from an oligonucleotide primer annealed to the DNA template of interest. Methods have been developed for the use of both single- and double-stranded templates. The chain termination reaction products were electrophoresed on urea-acrylamide gels and detected either by autoradiography (for radionuclide-labeled precursors) or by fluorescence (for fluorescent-labeled precursors). Recent improvements in mechanized reaction preparation, sequencing and analysis using the fluorescent detection method have permitted expansion in the number of sequences that can be determined per day (using machines such as the Catalyst 800 and the Applied Biosystems 377 or 373 DNA sequencer). Alternatively, cDNA inserts may be sequenced using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown, MA) along with Applied Biosystems 377 or 373 DNA Sequencing System.

The nucleotide sequence can be used in an assay to detect disorders associated with altered expression of HRAB18. The nucleotide sequence can be labeled by methods known in the art and added to a fluid or tissue sample from a patient under hybridizing conditions. After an incubation period, the sample is washed with a compatible fluid which optionally contains a dye (or other label requiring a developer) if the nucleotide has been labeled with an enzyme. After the compatible fluid is rinsed off, the

dye is quantitated and compared with a standard. If the amount of dye is significantly elevated, the nucleotide sequence has hybridized with the sample, and the assay indicates the presence of membrane trafficking disorders.

The nucleotide sequence for hrab18 can be used to construct hybridization probes for mapping that gene. The nucleotide sequence provided herein may be mapped to a particular chromosome or to specific regions of that chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include in situ hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries, flow-sorted chromosomal preparations, or artificial chromosome constructions YAC or P1 constructions. The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York City.

Fluorescent in situ hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of hrab18 on a physical chromosomal map and a specific disease (or predisposition to a specific disease) can help delimit the region of DNA associated with that genetic disease. The nucleotide sequence of the subject invention may be used to detect differences in gene sequence between normal and carrier or affected individuals.

Nucleotide sequences encoding HRAB18 may be used to produce purified HRAB18 using well known methods of recombinant DNA technology. Among the many publications that teach methods for the expression of genes after they have been isolated is Goeddel (1990) Gene Expression Technology, Methods and Enzymology, Vol 185, Academic Press, San Diego CA. Purification steps vary with the production process and the particular protein produced. Various methods for the isolation of the HRAB18 polypeptides may be accomplished by procedures well known in the art including those described in Deutscher M (1990) Methods in Enzymology, Vol 182, Academic Press, San Diego CA; and Scopes R (1982) Protein Purification: Principles and Practice, Springer-Verlag, New York City, both incorporated herein by reference.

HRAB18 may be expressed in a variety of host cells, either prokaryotic or eukaryotic. Host cells may be from the same species in which HRAB18 nucleotide sequences are endogenous or from a different species. Advantages of producing HRAB18 by recombinant DNA technology include obtaining adequate amounts of the protein for purification and the availability of simplified purification procedures.

Cells transformed with DNA encoding HRAB18 may be cultured under

conditions suitable for the expression of RAB proteins and recovery of the protein from the cell culture. HRAB18 produced by a recombinant cell may be secreted or may be contained intracellularly, depending on the hrab18 sequence and the genetic construction used. In general, it is more convenient to prepare recombinant proteins in secreted form.

In addition to recombinant production, fragments of HRAB18 may be produced by direct peptide synthesis using solid-phase techniques (Stewart et al (1969) Solid-Phase Peptide Synthesis, WH Freeman Co, San Francisco, CA; Merrifield J (1963) J Am Chem Soc 85:2149-2154. In vitro protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Foster City, CA) in accordance with the instructions provided by the manufacturer. Various fragments of HRAB18 may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

HRAB18 for antibody induction does not require biological activity; however, the protein must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at least five amino acid residues, preferably at least 10 amino acid residues. They should mimic a portion of the amino acid sequence of the protein and may contain the entire amino acid sequence of a small naturally occurring molecule such as HRAB18. Short stretches of HRAB18 may be fused with those of another protein such as keyhole limpet hemocyanin (KLH, Sigma, St Louis, MO) and the chimeric molecule used for antibody production.

Antibodies specific for HRAB18 may be produced by inoculation of an appropriate animal with the polypeptide or an antigenic fragment. An antibody is specific for HRAB18 if it is produced against an epitope of the polypeptide and binds to at least part of the natural or recombinant protein. Antibody production includes not only the stimulation of an immune response by injection into animals, but also analogous steps in the production of synthetic antibodies or other specific-binding molecules such as the screening of recombinant immunoglobulin libraries (Orlandi R et al (1989) Proc. Nat. Acad. Sci. USA 86:3833-3837, or Huse WD et al (1989) Science 256:1275-1281) or the in vitro stimulation of lymphocyte populations. Current technology (Winter G and Milstein C (1991) Nature 349:293-299) provides for a number of highly specific binding reagents based on the principles of antibody formation. These techniques may be adapted to produce molecules specifically binding HRAB18.

The present invention includes purified HRAB18 polypeptide from natural or recombinant sources, ie, cells transformed with recombinant nucleic acid molecules encoding HRAB18. Various methods for the isolation of the HRAB18 polypeptides may be accomplished by procedures well known in the art. For example, such polypeptides may be purified by immunoaffinity

chromatography by employing the antibodies provided by the present invention. Various other methods of protein purification well known in the art include those described in Deutscher M (1990) Methods in Enzymology, Vol 182, Academic Press, San Diego, CA; and Scopes R (1982) Protein Purification: Principles and Practice, Springer-Verlag, New York City, both incorporated herein by reference.

HRAB18 may be used to screen or design drugs that may be employed to regulate hormonal secretions or the expression of specific receptors of the pituitary that are associated with abnormal expression of HRAB18. Alternatively, HRAB18 itself may serve to control excessive hormonal secretion or to regulate the expression of specific receptors. Additionally, HRAB18 may serve similar functions in other neuronal tissues, particularly where secretory pathways play an important role in function.

HRAB18 as a bioactive agent or composition may be administered in a suitable therapeutic dose determined by any of several methodologies including clinical studies on mammalian species to determine maximal tolerable dose and on normal human subjects to determine safe dose. Additionally, the bioactive agent may be complexed with a variety of well established compounds or compositions which enhance stability or pharmacological properties such as half-life. It is contemplated that the therapeutic, bioactive composition may be delivered by intravenous infusion into the bloodstream or any other effective means which could be used for treating problems involving the altered expression or activity of RAB proteins.

The examples below are provided to illustrate the subject invention. These examples are provided by way of illustration and are not included for the purpose of limiting the invention.

**INDUSTRIAL APPLICABILITY**

**I Isolation of mRNA and Construction of cDNA Libraries**

Incyte clone 112352 was identified among the sequences of a human pituitary cDNA library constructed from a pooled sample of 21 whole, normal human pituitary glands from brains of Caucasian males and females with a range of ages from 16-70 years. Poly $A^+$ RNA was isolated using biotinylated oligo d(T) primer and streptavidin coupled to a paramagnetic particle (Promega Corp, Madison WI) and sent to Stratagene (La Jolla, CA).

Stratagene prepared the cDNA library using oligo d(T) priming. Synthetic adapter oligonucleotides were ligated onto the cDNA molecules enabling them to be inserted into the Uni-ZAP™ vector system (Stratagene). This allowed high efficiency unidirectional (sense orientation) lambda library construction and the convenience of a plasmid system with blue/white color selection to detect clones with cDNA insertions.

The quality of the cDNA library was screened using DNA probes, and then, the pBluescript® phagemid (Stratagene) was excised. This phagemid

allows the use of a plasmid system for easy insert characterization, sequencing, site-directed mutagenesis, the creation of unidirectional deletions and expression of fusion polypeptides. Subsequently, the custom-constructed library phage particles were infected into E. coli host strain XL1-Blue® (Stratagene). The high transformation efficiency of this bacterial strain increases the probability that the cDNA library will contain rare, under-represented clones. Alternative unidirectional vectors might include, but are not limited to, pcDNAI (Invitrogen, San Diego, CA) and pSHlox-1 (Novagen, Madison, WI).

**II Isolation of cDNA Clones**

The phagemid forms of individual cDNA clones were obtained by the in vivo excision process, in which XL1-BLUE was coinfected with an f1 helper phage. Proteins derived from both lambda phage and f1 helper phage initiated new DNA synthesis from defined sequences on the lambda target DNA and create a smaller, single-stranded circular phagemid DNA molecule that includes all DNA sequences of the pBluescript plasmid and the cDNA insert. The phagemid DNA was released from the cells and purified, then used to reinfect fresh bacterial host cells (SOLR, Stratagene Inc), where the double-stranded phagemid DNA was produced. Because the phagemid carries the gene for ß-lactamase, the newly transformed bacteria were selected on medium containing ampicillin.

Phagemid DNA was purified using the QIAWELL-8 Plasmid Purification System from QIAGEN® DNA Purification System (QIAGEN Inc, Chatsworth, CA). This technique provides a rapid and reliable high-throughput method for lysing the bacterial cells and isolating highly purified phagemid DNA. The DNA eluted from the purification resin was suitable for DNA sequencing and other analytical manipulations.

An alternate method of purifying phagemid has recently become available. It utilizes the Miniprep Kit (Catalog No. 77468, Advanced Genetic Technologies Corporation, Gaithersburg, MD). This kit is in the 96-well format and provides enough reagents for 960 purifications. Each kit is provided with a recommended protocol, which has been employed except for the following changes. First, the 96 wells are each filled with only 1 ml of sterile terrific broth with carbenicillin at 25 mg/L and glycerol at 0.4%. After the wells are inoculated, the bacteria are cultured for 24 hours and lysed with 60 µl of lysis buffer. A centrifugation step (2900 rpm for 5 minutes) is performed before the contents of the block are added to the primary filter plate. The optional step of adding isopropanol to TRIS buffer is not routinely performed. After the last step in the protocol, samples are transferred to a Beckman 96-well block for storage.

**III Sequencing of cDNA Clones**

The cDNA inserts from random isolates of the pituitary library were sequenced by the method of Sanger F. and AR Coulson (1975; J. Mol. Biol.

94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno NV) in combination with four Peltier Thermal Cyclers (PTC200 from MJ Research, Watertown MA) and Applied Biosystems 377 or 373 DNA Sequencing Systems (Perkin Elmer) and reading frame determined.

**IV Homology Searching of cDNA Clones and Deduced Proteins**

Each sequence so obtained was compared to sequences in GenBank using a search algorithm developed by Applied Biosystems Inc. and incorporated into the INHERIT™ 670 Sequence Analysis System. In this algorithm, Pattern Specification Language (developed by TRW Inc., Los Angeles, CA) was used to determine regions of homology. The three parameters that determine how the sequence comparisons run were window size, window offset, and error tolerance. Using a combination of these three parameters, the DNA database was searched for sequences containing regions of homology to the query sequence, and the appropriate sequences were scored with an initial value. Subsequently, these homologous regions were examined using dot matrix homology plots to distinguish regions of homology from chance matches. Smith-Waterman alignments of the protein sequence were used to display the results of the homology search.

Peptide and protein sequence homologies were ascertained using the INHERIT 670 Sequence Analysis System in a way similar to that used in DNA sequence homologies. Pattern Specification Language and parameter windows were used to search protein databases for sequences containing regions of homology which were scored with an initial value. Dot-matrix homology plots were examined to distinguish regions of significant homology from chance matches.

Alternatively, BLAST, which stands for Basic Local Alignment Search Tool, was used to search for local sequence alignments (Altschul SF (1993) J Mol Evol 36:290-300; Altschul, SF et al (1990) J Mol Biol 215:403-10). BLAST produces alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST is especially useful in determining exact matches or in identifying homologues. Although it is ideal for matches which do not contain gaps, it is inappropriate for performing motif-style searching. The fundamental unit of BLAST algorithm output is the high-scoring segment pair (HSP).

An HSP consists of two sequence fragments of arbitrary but equal lengths whose alignment is locally maximal and for which the alignment score meets or exceeds a threshold or cutoff score set by the user. The BLAST approach is to look for HSPs between a query sequence and a database sequence, to evaluate the statistical significance of any matches found, and to report only those matches which satisfy the user-selected threshold of significance. The parameter E establishes the statistically significant threshold for reporting database sequence matches. E is interpreted as the

upper bound of the expected frequency of chance occurrence of an HSP (or set of HSPs) within the context of the entire database search. Any database sequence whose match satisfies E is reported in the program output. An E greater than or equal to 25 usually indicates that a match is significant.

The nucleotide sequence for the entire coding region (included within SEQ ID NO:1) of the human homolog of the mouse RAB18, HRAB18, is shown in Figure 1.

BLAST results showed that the coding sequence of the clone of the subject invention had an E parameter value of 156 when compared with that of the mouse rab18 gene (GenBank accession numbers X80333 and LO4966). The coding sequence also shares HSP sequences with a human rab2 coding sequence (GenBank accession number M28213) with an E value of 54, and a human rab13 coding sequence (GenBank accession number X75593) with an E value of 51.

The coding sequence also shares HSP sequences with several clones in the LIFESEQ™ database (Incyte Pharmaceuticals, Palo Alto California) including Incyte clone 45334 derived from corneal stroma (E=37); Incyte clone 57291 derived from skeletal muscle (E=37); and Incyte clone 181288 derived from human placenta (E=36). All three tissue types are enervated. The presence of hrab18 related nucleotide sequences in these tissue types may result from the presence of nerve cells containing related RAB proteins involved in secretory vesicle trafficking. In addition, Incyte clone 269502 derived from a neuronal cell line (hNT) contains a coding sequence which shares high homology (87%) with the mouse rab18 gene.

**V Identification and Full Length Sequencing of the Genes**

The complete hrab18 nucleotide sequence was obtained from Incyte clone 112352. The sequence for the full length hrab18 gene was translated, and the putative in-frame translation is shown in Figure 1. When all three possible predicted translations of the sequence were searched against protein databases such as SwissProt and PIR, no exact matches were found to the possible translations of HRAB18. Figure 2 shows the comparison of the HRAB18 amino acid sequence with GenBank mouse RAB18. The substantial region of homology among these molecules encompasses the whole length of the molecule with only two out of 207 residues not conserved.

**VI Antisense analysis**

Knowledge of the cDNA sequence of the hrab18 gene will enable its use in antisense technology in the investigation of gene function. Oligonucleotides, genomic or cDNA fragments comprising the antisense strand of hrab18 are used either in vitro or in vivo to inhibit expression of the protein. Such technology is now well known in the art, and probes are designed at various locations along the nucleotide sequence. By transfection of cells or whole test animals with such antisense sequences,

the gene of interest are effectively turned off. The function of the gene is ascertained by observing behavior at the cellular, tissue or organismal level (e.g. changes in secretory pathways, lethality, loss of differentiated function, changes in morphology, for example).

In addition to using sequences constructed to interrupt transcription of the open reading frame, modifications of gene expression are obtained by designing antisense sequences to intron regions, promoter/enhancer elements, or even to trans-acting regulatory genes. Similarly, inhibition is achieved using Hogeboom base-pairing methodology, also known as "triple helix" base pairing.

**VII Expression of HRAB18**

Expression of HRAB18 is accomplished by subcloning the cDNAs into appropriate expression vectors and transfecting the vectors into appropriate expression hosts. In this particular case, the cloning vector used in the generation of the full length clone also provides for expression of the included hrab18 sequence in E. coli. Upstream of the cloning site, this vector contains a promoter for ß-galactosidase, followed by sequence containing the amino-terminal Met and the subsequent 7 residues of ß-galactosidase. Immediately following these eight residues is an engineered bacteriophage promoter useful for artificial priming and transcription and a number of unique restriction sites, including Eco RI, for cloning.

Induction of the isolated, transfected bacterial strain with IPTG using standard methods will produce a fusion protein corresponding to the first seven residues of ß-galactosidase, about 15 residues of "linker", and the peptide encoded within the cDNA. Since cDNA clone inserts are generated by an essentially random process, there is one chance in three that the included cDNA will lie in the correct frame for proper translation. If the cDNA is not in the proper reading frame, it can be obtained by deletion or insertion of the appropriate number of bases by well known methods including in vitro mutagenesis, digestion with exonuclease III or mung bean nuclease, or oligonucleotide linker inclusion.

The hrab18 cDNA can be shuttled into other vectors known to be useful for expression of protein in specific hosts. Oligonucleotide amplimers containing cloning sites as well as a segment of DNA sufficient to hybridize to stretches at both ends of the target cDNA (25 bases) can be synthesized chemically by standard methods. These primers can then used to amplify the desired gene segments by PCR. The resulting new gene segments can be digested with appropriate restriction enzymes under standard conditions and isolated by gel electrophoresis. Alternately, similar gene segments can be produced by digesting the cDNA with appropriate restriction enzymes and filling in the missing gene segments with chemically synthesized oligonucleotides. Segments of the coding sequence from more

than one gene can be ligated together and cloned in appropriate vectors to optimize expression of the recombinant sequence.

Suitable expression hosts for such chimeric molecules include but are not limited to mammalian cells such as Chinese Hamster Ovary (CHO) and human 293 cells, insect cells such as Sf9 cells, yeast cells such as Saccharomyces cerevisiae, and bacteria such as E. coli. For each of these cell systems, a useful expression vector includes an origin of replication to allow propagation in bacteria and a selectable marker such as the ß-lactamase antibiotic resistance gene to allow selection in bacteria. In addition, the vectors include a second selectable marker such as the neomycin phosphotransferase gene to allow selection in transfected eukaryotic host cells. Vectors for use in eukaryotic expression hosts require RNA processing elements such as 3' polyadenylation sequences if such are not part of the cDNA of interest.

Additionally, the vector may contain promoters or enhancers which increase gene expression. Such promoters are host specific and include MMTV, SV40, or metallothionine promoters for CHO cells; trp, lac, tac or T7 promoters for bacterial hosts, or alpha factor, alcohol oxidase or PGH promoters for yeast. Transcription enhancers, such as the rous sarcoma virus (RSV) enhancer, may be used in mammalian host cells. Once homogeneous cultures of recombinant cells are obtained through standard culture methods, large quantities of recombinantly produced HRAB18 can be recovered from the conditioned medium and analyzed using chromatographic methods known in the art.

**VIII Isolation of Recombinant HRAB18**

HRAB18 is expressed as a chimeric protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of a cleavable linker sequence such as Factor XA or enterokinase (Invitrogen) between the purification domain and the hrab18 sequence provides for purification of HRAB18 from the fusion protein.

**IX Production of HRAB18 Specific Antibodies**

Two approaches are utilized to raise antibodies to HRAB18, and each approach is useful for generating either polyclonal or monoclonal antibodies. In one approach, denatured protein from the reverse phase HPLC separation is obtained in quantities up to 75 mg. This denatured protein is used to immunize mice or rabbits using standard protocols; about 100 micrograms are adequate for immunization of a mouse, while up to 1 mg are used to immunize a rabbit. For identifying mouse hybridomas, the denatured

protein is radioiodinated and used to screen potential murine B-cell hybridomas for those which produce antibody. This procedure requires only small quantities of protein, such that 20 mg is sufficient for labeling and screening several thousand clones.

In the second approach, the amino acid sequence of HRAB18, as deduced from translation of the cDNA, is analyzed to determine regions of high immunogenicity. Oligopeptides comprising appropriate hydrophilic regions, as shown in Figure 3, are synthesized and used in suitable immunization protocols to raise antibodies. Analysis to select appropriate epitopes is described by Ausubel FM et al., supra. The optimal amino acid sequences for immunization are at the C-terminus, the N-terminus and those intervening, hydrophilic regions of the polypeptide which are likely to be exposed to the external environment when the protein is in its natural conformation.

Typically, selected peptides, about 15 residues in length, are synthesized using an Applied Biosystems Peptide Synthesizer Model 431A using fmoc-chemistry and coupled to keyhole limpet hemocyanin (KLH, Sigma) by reaction with M-maleimidobenzoyl-N- hydroxysuccinimide ester (MBS; Ausubel FM et al, supra). If necessary, a cysteine is introduced at the N-terminus of the peptide to permit coupling to KLH. Rabbits are immunized with the peptide-KLH complex in complete Freund's adjuvant. The resulting antisera are tested for antipeptide activity by binding the peptide to plastic, blocking with 1% BSA, reacting with antisera, washing and reacting with labeled (radioactive or fluorescent), affinity purified, specific goat anti-rabbit IgG.

Hybridomas are prepared and screened using standard techniques. Hybridomas of interest are detected by screening with labeled HRAB18 to identify those fusions producing the monoclonal antibody with the desired specificity. In a typical protocol, wells of plates (FAST; Becton-Dickinson, Palo Alto, CA) are coated with affinity purified, specific rabbit-anti-mouse (or suitable anti-species Ig) antibodies at 10 mg/ml. The coated wells are blocked with 1% BSA, washed and exposed to supernatants from hybridomas. After incubation the wells are exposed to labeled HRAB18, 1 mg/ml. Clones producing antibodies will bind a quantity of labeled HRAB18 which is detectable above background. Such clones are expanded and subjected to 2 cycles of cloning at limiting dilution (1 cell/3 wells). Cloned hybridomas are injected into pristane mice to produce ascites, and monoclonal antibody is purified from mouse ascitic fluid by affinity chromatography on Protein A. Monoclonal antibodies with affinities of at least $10^8$ $M^{-1}$, preferably $10^9$ to $10^{10}$ or stronger, will be made by standard procedures as described in Harlow and Lane (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY; and in Goding (1986) Monoclonal Antibodies: Principles and

Practice, Academic Press, New York City, both incorporated herein by reference.

**X Diagnostic Test Using HRAB18 Specific Antibodies**

Particular HRAB18 antibodies are useful for the diagnosis of disorders which are characterized by differences in the amount or distribution of HRAB18 in the pituitary or other neuronally derived cells. Diagnostic tests for HRAB18 include methods utilizing the antibody and a label to detect HRAB18 in human bodily fluids, tissues or extracts of such tissues. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and have been reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, chromogenic agents, magnetic particles and the like. Patents teaching the use of such labels include US Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in US Patent No. 4,816,567, incorporated herein by reference.

A variety of protocols for measuring soluble or membrane-bound HRAB18, using either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on HRAB18 is preferred, but a competitive binding assay may be employed. These assays are described, among other places, in Maddox, DE et al (1983, J Exp Med 158:1211).

**XI Purification of Native HRAB18 Using Specific Antibodies**

Naturally occurring or recombinant HRAB18 is purified by immunoaffinity chromatography using antibodies specific for HRAB18. In general, an immunoaffinity column is constructed by covalently coupling the anti-HRAB18 antibody to an activated chromatographic resin.

Polyclonal immunoglobulins are prepared from immune sera either by precipitation with ammonium sulfate or by purification on immobilized Protein A (Pharmacia LKB Biotechnology, Piscataway, NJ). Likewise, monoclonal antibodies are prepared from mouse ascites fluid by ammonium sulfate precipitation or chromatography on immobilized Protein A. Partially purified immunoglobulin is covalently attached to a chromatographic resin such as CnBr-activated Sepharose (Pharmacia LKB Biotechnology). The antibody is coupled to the resin, the resin is

blocked, and the derivative resin is washed according to the manufacturer's instructions.

Such immunoaffinity columns are utilized in the purification of HRAB18 by preparing a fraction from cells containing HRAB18 in a soluble form. This preparation is derived by solubilization of the whole cell or of a subcellular fraction obtained via differential centrifugation by the addition of detergent or by other methods well known in the art. Alternatively, soluble HRAB18 containing a signal sequence is secreted in useful quantity into the medium in which the cells are grown.

A soluble HRAB18-containing preparation is passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of RAB proteins (eg, high ionic strength buffers in the presence of detergent). Then, the column is eluted under conditions that disrupt antibody/HRAB18 binding (e.g., a buffer of pH 2-3 or a high concentration of a chaotrope such as urea or thiocyanate ion), and HRAB18 is collected.

**XII HRAB18 Localization and Activity**

HRAB18 may be localized in neuronal cells, particularly pituitary cells, in the following manner. First, either naturally-occurring HRAB18 or HRAB18 purified from E. Coli expressing the protein with its C-terminus prenylated in vitro is obtained. The prenylation allows HRAB18 to become localized to cellular compartments, such as the late endosomes, the trans Golgi network, the cis Golgi network, or the endoplasmic reticulum, within a cell. The prenylated HRAB18 is added to a cell-free system, such as one where the plasma membrane has been solubilized by digitonin. The HRAB18 is added at a concentration so as to observe specific binding of HRAB18 to specific cellular compartments. The localization is monitored with radiolabeled antibodies.

Once HRAB18 is localized to a specific cellular compartment, cell-free reconstitution studies may be performed to investigate its function. For example, a cell-free system can be developed that is capable of measuring vesicular transport from the endoplasmic reticulum to the trans Golgi network. Preferably, this cell free system is depleted of naturally occurring HRAB18 to allow study of the effect of cell free systems lacking RAB18 on vesicular transport. The concentration of HRAB18 is gradually increased to recover HRAB18 activity in vesicular transport. This method is used to test HRAB18 derivatives for biological activity.

**XIII Drug Screening**

HRAB18 or host cells containing HRAB18 are used to screen compounds that may affect vesicle trafficking by HRAB18, its isoforms or even other RAB proteins. The polypeptide or fragment employed in such a test is used in a cell free system or located intracellularly. One method of compound

screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide of fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, alterations in vesicular transport of specific peptides or neurotransmitters.

Thus, the present invention provides methods of screening for test compounds which can affect HRAB18 activity. These methods comprise contacting a compound with HRAB18 and assaying for the presence of a complex between the compound and HRAB18 by methods well known in the art. After suitable incubation, free compound is separated from that in bound form, and the amount of bound compound is a measure of its ability to interfere in the regular functioning of HRAB18.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to HRAB18, and described in European Patent 84/03564, incorporated herein by reference.

Competitive drug screening assays in which neutralizing antibodies capable of binding HRAB18 specifically compete with a test compound for binding to HRAB18 are used to determine compounds which specifically bind HRAB18. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic determinants with HRAB18.

**XIV Rational Drug Design**

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact, including nonhydrolyzable analogs of GTP, for example. Any of these examples can be used to fashion drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide in vivo (Hodgson J (1991) Bio/Technology 9:19-21, incorporated herein by reference).

In one approach, the three-dimensional structure of a protein of interest, or of a protein-inhibitor complex, is determined by x-ray crystallography, by computer modeling or, most typically, by a combination of the two approaches. Both the shape and charges of the polypeptide are ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide is gained by modeling based on the structure of homologous proteins. In both cases, relevant structural information is used to design analogous RAB-like molecules or to identify efficient inhibitors. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton S and Wells JA (1992) Biochemistry 31:7796-7801 or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda SB et al (1993) J Biochem 113:742-746, incorporated herein by reference.

It is also possible to isolate a target-specific antibody, selected

by functional assay, as described above, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids is an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides act as the pharmacore.

HRAB18 is used to perform such analytical studies as X-ray crystallography. In addition, knowledge of the HRAB18 amino acid sequence provided herein will provide guidance to those employing computer modeling techniques in place of or in addition to x-ray crystallography.

**XV Use and Administration of Drugs**

Numerous diseases have been associated with the altered secretion of hormones or the abnormal recycling of surface receptors in secretory tissue, particularly pituitary tissue. For example, most cases of dwarfism are caused by a deficiency of all anterior pituitary secretion, while gigantism results from excessive activity and secretion of GH by somatropic cells. All these diseases may be associated with an abnormal regulation of vesicle transport, fusion and targeting associated with the altered expression of HRAB18. In addition, since HRAB18 may play a role in endocytosis, altered expression of HRAB18 may result in disorders related to the abnormal recycling of receptors. Since HRAB18 appears to regulate vesicular transport between intracellular compartments, compounds that bind HRAB18 may be used therapeutically to treat abnormal secretion of pituitary hormones or abnormal levels of receptors on the pituitary cell surface. Alternatively, these compounds may regulate the abnormal secretion of neurotransmitters from neuronal cells. Furthermore, HRAB18 itself may be administered to treat a disorder associated with the altered expression of HRAB18.

Therapeutic compounds are formulated in a nontoxic, inert, pharmaceutically acceptable aqueous carrier medium preferably at a pH of about 5 to 8, more preferably 6 to 8, although the pH may vary according to the characteristics of the formulation and its administration. Characteristics such as solubility of the molecule, half-life and antigenicity/immunogenicity will aid in defining an effective carrier. Recombinant, organic or synthetic molecules resulting from drug design may be equally effective in particular situations.

Therapeutic compounds are delivered by known routes of administration including but not limited to topical creams and gels; transmucosal spray and aerosol, transdermal patch and bandage; injectable, intravenous and lavage formulations; and orally administered liquids and pills,

particularly formulated to resist stomach acid and enzymes. The particular formulation, exact dosage, and route of administration will be determined by the attending physician and will vary according to each specific situation.

Such determinations are made by considering multiple variables such as the condition to be treated, the therapeutic compound to be administered, and the pharmacokinetic profile of the particular therapeutic compound. Additional factors which may be taken into account include disease state (e.g. severity) of the patient, age, weight, gender, diet, time of administration, drug combination, reaction sensitivities, and tolerance/response to therapy. Long acting therapeutic compound formulations might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular therapeutic HRAB18.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see US Patent Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different uses of therapeutic compounds and that administration targeting a tissue or organ may necessitate delivery in a specific manner.

All publications and patents mentioned in the above specification are herein incorporated by reference. The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. Indeed, various modifications of the above described modes for carrying out the invention which are readily apparent to those skilled in the field of molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: INCYTE PHARMACEUTICALS, INC.

(ii) TITLE OF INVENTION: HUMAN HOMOLOG OF A MOUSE RAB 18 GENE

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: INCYTE PHARMACEUTICALS, INC.
(B) STREET: 3174 Porter Drive
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) PCT APPLICATION NUMBER: PCT/US96/10699
(B) FILING DATE: 21-JUN-1996
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/000,377
(B) FILING DATE: 21-JUN-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/569,062
(B) FILING DATE: 06-DEC-1995

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Glaister, Debra J.
(B) REGISTRATION NUMBER: 33888
(C) REFERENCE/DOCKET NUMBER: PF-0043 PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 415-855-0555
(B) TELEFAX: 415-845-4166

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1148 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Pituitary

(B) CLONE: 112352

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CGCACCCGGG CGGCCAGCTG GGCTCGGAGC GGAACGGGGT CAGGATGGAC GAGGACGTGC      60
TAACCACCCT GAAGATCCTC ATCATCGGCG AGAGTGGGGT GGGCAAGTCC AGCCTGCTCT     120

TGAGGTTCAC AGATGATACG TTTGATCCAG AACTTGCAGC AACAATAGGT GTTGACTTTA     180

AGGTGAAAAC AATTTCAGTG GATGGAAATA AGGCTAAACT TGCAATATGG GATACTGCTG     240

GTCAAGAGAG GTTTAGAACA TTAACTCCCA GCTATTATAG AGGTGCACAG GGTGTTATAT     300

TAGTTTATGA TGTCACAAGA AGAGATACAT TTGTTAAACT GGATAACTGG TTAAATGAAT     360

TGGAAACATA CTGTACAAGA AATGACATAG TAAACATGCT AGTTGGAAAT AAAATCGATA     420

AGGAAAATCG TGAAGTCGAT AGAAATGAAG GCCTGAAATT TGCACGAAAG CATTCCATGT     480

TATTTATAGA GGCAAGTGCA AAAACCTGTG ATGGTGTACA ATGTGCCTTT GAAGAACTTG     540

TTGAAAAGAT CATTCAGACC CCTGGACTGT GGGAAAGTGA GAACCAGAAT AAAGGAGTCA     600

AACTGTCACA CAGGGAAGAA GGCCAAGGAG GAGGAGCCTG TGGTGGTTAT TGCTCTGTGT     660

TATAAACTCT GGGAAATTCC ATCTCTTGCA TATTTGATCA GATAGTGACA TCTTTCTGTA     720

TATAAACTCT TTAACCTGCT ATTTTAGGGA CCTTGCAGTT TGCACATAAT TGTTTTATAT     780

CATAGCAGTA AATATTTGCA AGAAATCCCA CTCATCGACC CCGGGTAAAA TGTTATGGTA     840

AGCATGCACA GTTTGCAGTC TACAGTTTTT TTATGTAGCA CCAAATAGGT GTACCTTTAT     900

AAGTACATTC AATTTTATGA TTTACATTTA TCATGTAATT TTTAAAAAAA TCCATCTATC     960

TAGGATATGT TGATACAAAG TCTGCTTTTG CTATTCTTTT TGCTTAAATA CTCCTATCAT    1020

TTTCTGAATT ACTTGGTATT TAGAACTCCT AGCACCACGG GGAAGAATAG AGGTATCATC    1080

AAACGTGGCA AATTTTCTTT CAGGAATAAT AAAGAGCATG ATTCCACAGC CAAAAAAAAA    1140

AAAAAAAA                                                             1148
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 206 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Glu Asp Val Leu Thr Thr Leu Lys Ile Leu Ile Ile Gly Glu
 1               5                  10                  15

Ser Gly Val Gly Lys Ser Ser Leu Leu Leu Arg Phe Thr Asp Asp Thr
            20                  25                  30
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Phe Asp Pro Glu Leu Ala Ala Thr Ile Gly Val Asp Phe Lys Val Lys
          35                  40                  45

Thr Ile Ser Val Asp Gly Asn Lys Ala Lys Leu Ala Ile Trp Asp Thr
      50                  55                  60

Ala Gly Gln Glu Arg Phe Arg Thr Leu Thr Pro Ser Tyr Tyr Arg Gly
 65                  70                  75                  80

Ala Gln Gly Val Ile Leu Val Tyr Asp Val Thr Arg Arg Asp Thr Phe
                 85                  90                  95

Val Lys Leu Asp Asn Trp Leu Asn Glu Leu Glu Thr Tyr Cys Thr Arg
            100                 105                 110

Asn Asp Ile Val Asn Met Leu Val Gly Asn Lys Ile Asp Lys Glu Asn
        115                 120                 125

Arg Glu Val Asp Arg Asn Glu Gly Leu Lys Phe Ala Arg Lys His Ser
    130                 135                 140

Met Leu Phe Ile Glu Ala Ser Ala Lys Thr Cys Asp Gly Val Gln Cys
145                 150                 155                 160

Ala Phe Glu Glu Leu Val Glu Lys Ile Ile Gln Thr Pro Gly Leu Trp
                165                 170                 175

Glu Ser Glu Asn Gln Asn Lys Gly Val Lys Leu Ser His Arg Glu Glu
            180                 185                 190

Gly Gln Gly Gly Gly Ala Cys Gly Gly Tyr Cys Ser Val Leu
        195                 200                 205
```

CLAIMS

1. A purified polynucleotide comprising a nucleic acid sequence encoding the polypeptide having the sequence as depicted in SEQ ID NO:2, or its complement.

2. The polynucleotide of Claim 1 wherein the nucleic acid sequence comprises the sequence shown in SEQ ID NO:1 from nucleotide 45 to nucleotide 664.

3. A purified polynucleotide comprising the nucleic acid sequence of SEQ ID NO:1 from nucleotide 1 to nucleotide 44.

4. A purified polynucleotide comprising the nucleic acid sequence of SEQ ID NO:1 from nucleotide 665 to 1148.

5. An expression vector comprising the polynucleotide of Claim 1.

6. A host cell comprising the expression vector of Claim 5.

7. An antisense molecule comprising a polynucleotide sequence complementary to at least a portion of the polynucleotide of Claim 2.

8. A method for producing a polypeptide comprising the sequence as depicted in SEQ ID NO:2, said method comprising:

a) culturing the host cells of Claim 6 under conditions suitable for the expression of the polypeptide, and

b) recovering said polypeptide from the cell culture.

9. Purified HRAB18 having the amino acid sequence as depicted in SEQ ID NO:2.

10. An antibody specific for the purified polypeptide of Claim 9.

11. A method of screening a plurality of test compounds for binding to the polypeptide of Claim 9, or a portion thereof, said method comprising the steps of:

a) providing a plurality of test compounds;

b) combining the polypeptide of Claim 9, or a portion thereof, with each of the test compounds for a time sufficient to allow binding under suitable conditions; and

c) detecting binding of the polypeptide of Claim 9 or a fragment thereof, to each of the plurality of compounds, thereby identifying the compounds which specifically bind the polypeptide of Clain 9 or a fragment thereof.

12. A diagnostic test for the detection of nucleic acid sequences encoding HRAB18 in a biological sample, comprising the steps of:

a) combining the biological sample with a polynucleotide which comprises the nucleic acid sequence of SEQ ID NO:1, or a fragment thereof, under conditions suitable for the formation of a nucleic acid hybridization complex between the nucleic acid sequence of SEQ ID NO:1 and a complementary nucleic acid sequence in said sample,

b) detecting said hybridization complex, and

c) comparing the amount of said hybridization complex with a standard wherein the presence of an abnormal level of said hybridization complex correlates positively with a condition associated with altered expression of HRAB18.

13. A diagnostic test for the detection of nucleotide sequences encoding HRAB18 in a biological sample, comprising the steps of:

a)combining the biological sample with polymerase chain reaction primers under conditions suitable for nucleic acid amplification, wherein said primers comprise fragments from the nucleotide sequence of SEQ ID NO:1;

b) detecting amplified nucleotide sequences; and

c) comparing the amount of amplified nucleotide sequences in said biological sample with a standard thereby determining whether the amount of said nucleotide sequence varies from said standard, wherein the presence of an abnormal level of said nucleotide sequence correlates positively with a condition associated with altered expression of HRAB18..

```
ATG GAC GAG GAC GTG CTA ACC ACC CTG AAG ATC CTC ATC ATC GGC GAG          48
Met Asp Glu Asp Val Leu Thr Thr Leu Lys Ile Leu Ile Ile Gly Glu
  1               5                  10                  15

AGT GGG GTG GGC AAG TCC AGC CTG CTC TTG AGG TTC ACA GAT GAT ACG          96
Ser Gly Val Gly Lys Ser Ser Leu Leu Leu Arg Phe Thr Asp Asp Thr
            20                  25                  30

TTT GAT CCA GAA CTT GCA GCA ACA ATA GGT GTT GAC TTT AAG GTG AAA         144
Phe Asp Pro Glu Leu Ala Ala Thr Ile Gly Val Asp Phe Lys Val Lys
        35                  40                  45

ACA ATT TCA GTG GAT GGA AAT AAG GCT AAA CTT GCA ATA TGG GAT ACT         192
Thr Ile Ser Val Asp Gly Asn Lys Ala Lys Leu Ala Ile Trp Asp Thr
    50                  55                  60

GCT GGT CAA GAG AGG TTT AGA ACA TTA ACT CCC AGC TAT TAT AGA GGT         240
Ala Gly Gln Glu Arg Phe Arg Thr Leu Thr Pro Ser Tyr Tyr Arg Gly
65                  70                  75                  80

GCA CAG GGT GTT ATA TTA GTT TAT GAT GTC ACA AGA AGA GAT ACA TTT         288
Ala Gln Gly Val Ile Leu Val Tyr Asp Val Thr Arg Arg Asp Thr Phe
                85                  90                  95

GTT AAA CTG GAT AAC TGG TTA AAT GAA TTG GAA ACA TAC TGT ACA AGA         336
Val Lys Leu Asp Asn Trp Leu Asn Glu Leu Glu Thr Tyr Cys Thr Arg
            100                 105                 110

AAT GAC ATA GTA AAC ATG CTA GTT GGA AAT AAA ATC GAT AAG GAA AAT         384
Asn Asp Ile Val Asn Met Leu Val Gly Asn Lys Ile Asp Lys Glu Asn
        115                 120                 125

CGT GAA GTC GAT AGA AAT GAA GGC CTG AAA TTT GCA CGA AAG CAT TCC         432
Arg Glu Val Asp Arg Asn Glu Gly Leu Lys Phe Ala Arg Lys His Ser
    130                 135                 140

ATG TTA TTT ATA GAG GCA AGT GCA AAA ACC TGT GAT GGT GTA CAA TGT         480
Met Leu Phe Ile Glu Ala Ser Ala Lys Thr Cys Asp Gly Val Gln Cys
145                 150                 155                 160

GCC TTT GAA GAA CTT GTT GAA AAG ATC ATT CAG ACC CCT GGA CTG TGG         528
Ala Phe Glu Glu Leu Val Glu Lys Ile Ile Gln Thr Pro Gly Leu Trp
                165                 170                 175

GAA AGT GAG AAC CAG AAT AAA GGA GTC AAA CTG TCA CAC AGG GAA GAA         576
Glu Ser Glu Asn Gln Asn Lys Gly Val Lys Leu Ser His Arg Glu Glu
            180                 185                 190

GGC CAA GGA GGA GGA GCC TGT GGT GGT TAT TGC TCT GTG TTA TAA             621
Gly Gln Gly Gly Gly Ala Cys Gly Gly Tyr Cys Ser Val Leu  *
        195                 200                 205
```

**FIGURE 1**


MDEDVLTTLKILIIGESGVGKSSLLLRFTD Majority
10 20 30
1 MDEDVLTTLKILIIGESGVGKSSLLLRFTD 112352.aa
1 MDEDVLTTLKILIIGESGVGKSSLLLRFTD MUSRAB18P.AA
DTFDPELAATIGVDFKVKTISVDGNKAKLA Majority
40 50 60
31 DTFDPELAATIGVDFKVKTISVDGNKAKLA 112352.aa
31 DTFDPELAATIGVDFKVKTISVDGNKAKLA MUSRAB18P.AA
IWDTAGQERFRTLTPSYYRGAQGVILVYDV Majority
70 80 90
61 IWDTAGQERFRTLTPSYYRGAQGVILVYDV 112352.aa
61 IWDTAGQERFRTLTPSYYRGAQGVILVYDV MUSRAB18P.AA
TRRDTFVKLDNWLNELETYCTRNDIVNMLV Majority
100 110 120
91 TRRDTFVKLDNWLNELETYCTRNDIVNMLV 112352.aa
91 TRRDTFVKLDNWLNELETYCTRNDIVNMLV MUSRAB18P.AA
GNKIDKENREVDRNEGLKFARKHSMLFIEA Majority
130 140 150
121 GNKIDKENREVDRNEGLKFARKHSMLFIEA 112352.aa
121 GNKIDKENREVDRNEGLKFARKHSMLFIEA MUSRAB18P.AA
SAKTCDGVQCAFEELVEKIIQTPGLWESEN Majority
160 170 180
151 SAKTCDGVQCAFEELVEKIIQTPGLWESEN 112352.aa
151 SAKTCDGVQCAFEELVEKIIQTPGLWESEN MUSRAB18P.AA
QNKGVKLSHREEGQGGGACGGYCSVL- Majority
190 200
181 QNKGVKLSHREEGQGGGACGGYCSVL 112352.aa
181 QNKGVKLSHREESRGGGACGGYCSVL. MUSRAB18P.AA

**FIGURE 2**

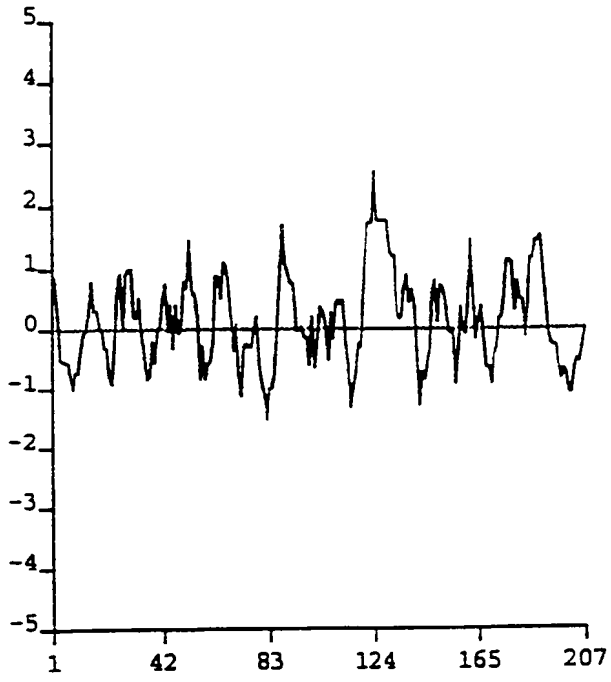


**FIGURE 3**

# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 96/10699

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/47 C07K16/18 C12Q1/00 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | GENE, vol. 132, no. 2, 15 October 1993, AMSTERDAM NL, pages 273-278, XP002015898 HELEN YU ET AL.: "Gene cloning and characterization of a GTP-binding Rab protein from mouse pituitary AtT-20 cells" cited in the application see abstract see page 274, left-hand column, paragraph 2 - page 278, left-hand column, line 1; figure 2 --- -/-- | 1-13 |

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 15 October 1996 | 25. 10. 96 |

| Name and mailing address of the ISA | Authorized officer |
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| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax (+31-70) 340-3016 | Montero Lopez, B |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | JOURNAL OF CELL SCIENCE, vol. 107, no. 12, December 1994, pages 3437-3448, XP000604112 ANNE LÜTCKE ET AL.: "Cloning and subcellular localization of a novel rab proteins reveals polarized and cell type-specific expression" see page 3438, left-hand column, paragraph 6 - right-hand column, paragraph 1 see page 3439, right-hand column, paragraph 1 - paragraph 2; figure 1 --- | 1-13 |
| A | JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 21, 25 July 1989, MD US, pages 12394-12401, XP002015899 A. ZAHRAOUI ET AL.: "The human Rab genes encode a family of GTP-binding proteins related to yeast YPT1 and SEC4 products involved in secretion" --- | 1-13 |
| A | EMBL Database Entry HS79235; Accession number T64792; 6 March 1995; HILLIER L. ET AL.: "The WashU-Merck EST Project" XP002015900 ----- | 1-4 |

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